Proceedings of the Fourth Annual Aquatic Toxicity Workshop November 8-10, 1977 Bayshore Inn, Vancouver, B.C.

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Editors

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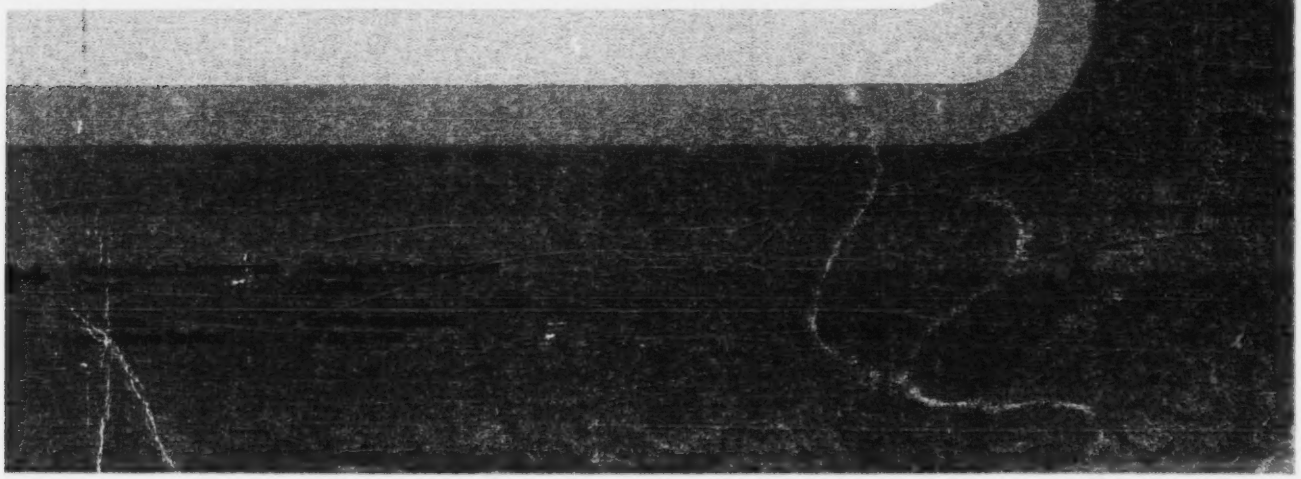

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Fisheries and Marine Service

Technical Report 818

October 1978

PROCEEDINGS OF THE FOURTH ANNUAL AQUATIC TOXICITY WORKSHOP

November 8-10, 1977, Bayshore Inn,

Vancouver, B.C.

J.C. Davis, G.L. Greer and I.K. Birtwell

Editors

Sponsored by Environment Canada Fisheries and Marine Service

and

Environmental Protection Service

ABSTRACT OF PROCEEDINGS

The proceedings of the Fourth Annual Aquatic Toxicity Workshop, held November 8-10, 1977, in Vancouver, B.C., consists of 10 complete papers, six abstracts only, and discussions from three problem-oriented workshop sessions. More than half the papers presented dealt with bioassays in varied form: field (*in situ*), laboratory, developmental studies using *Daphnia* and larval herring, toxicity of copper-nickel mixtures, pharmokinetics and toxicity assessment, and marine invertebrate species diversity. The toxic substances used in the bioassays included phenol, oil refinery effluent, fuel oil, pulp mill effluent, and sewage. Topics covered by other papers were avoidance behaviour of salmonids to suspended sediments, a review of experimental ecosystems for evaluating effects of contaminants in aquatic ecosystems, and approaches to aquatic contaminant surveillance.

The problems presented in the workshop discussion sessions were of a practical nature and represented current concerns of aquatic toxicologists. In one problem, participants were required to perform the function of a regulatory agency and review an application for estuarine discharge of effluent from a large kraft pulp mill. Another problem required the design of a study to assess the purported deleterious impact on a salmonid-bearing stream receiving a discharge continuously pumped from a copper mine flooded by an underground body of water. The third problem addressed the state of the art and future challenges in aquatic toxicology. Synopses of the discussion resulting from the workshop sessions are presented in an Appendix to the Proceedings.

Key words: Aquatic, toxicology, bioassay, pollutants, contaminants, ecosystems, toxicity assessment.

RESUMÉ DES COMPTES RENDUS

Les comptes-rendus de la quatrième réunion sur la toxicité dans le milieu aquatique, tenue à Vancouver, C.B., du 8 au 10 Novembre, consistent en 10 communications complètes, 6 resumés et les discussions aux 3 séances d'études concernant des problèmes à resoudre. Plus de la moitié des communications presentées traitaient de differentes sortes d'essais biologiques: sur le terrain (in situ), au laboratoire, des études sur la croissance en utilisant la *Daphnia* et les larves de harengs, la toxicité des mélanges de cuivre et de nickel, la pharmacocinétique et l'évaluation de la toxicité, el la varieté des espèces d'invertébrés marins. Les matières toxiques utilisées dans les essais biologiques comprenaient le phénol, les effluents des raffineries de pétrole, le mazout, les effluents des usines de pâte à papier et les égouts. Les sujets couverts par les autres communications conceraient la réponse d'évitement des salmonidés aux sediments en suspension, une revue critique des écosystèmes experimentaux servant à évaluer les effects des contaminants sur les écosystèmes aquatiques et les façons d'exercer la surveillance des contaminants dans le milieu aquatique.

Les problèmes présentés aux discussions des séances d'études avaient un charactère pratique et reflétaient les préocupations des toxicologistes du milieu aquatique. Dans un problème, on a demandé aux participants de remplir

la fonction d'une agence régulatrice et d'examiner une demande pour déverser dans l'estuaire les effluents d'une grande usine de pâte à papier (kraft). Dans un autre problème, il s'agissait d'élaborer une étude pour evaluer un effect, soi disant nuisible, qui se produit dans des conrs à saumon recevant une décharge continue (pompée) d'une mine sousterraine de cuivre, submergée par un cour d'eau. Le troisième problème traitait de l'état des connaisances actuelles et des futures demandes en toxicologie du milieu aquatique. Les résumés des discussions résultant des séances d'études sont presentés dans l'annexe des comptes-rendus.

Mots clefs: Aquatique, toxicologie, essai biologique, polluants, contaminant, écosystèmes, evaluation de la toxicité.

Preface

The Aquatic Toxicity Workshop had its beginnings in 1974 at the Freshwater Institute in Winnipeg, Manitoba. The event has continued annually with successive meetings being held in Rexdale, Ontario, Halifax, Nova Scotia and, most recently, in Vancouver, B.C.

Organization has always been somewhat informal and the meeting has grown out of a recognition amongst aquatic toxicologists and persons working in the environmental field, of a need for a Canadian forum for the regular exchange of new ideas and information. Early meetings dealt mainly with toxicity test procedures and regulatory test protocols although the perspective of the group appears to be widening with time. This is probably related to expansion of knowledge in the field and increasing participation of more diverse groups from both government and the private sector.

Sponsorship, organization and publication of proceedings has traditionally been the task of whatever group volunteers to organize the meeting in a given year. Details of past meetings can be obtained from:

First Annual Aquatic Toxicity Workshop (1974)
Dr. J. Klavercamp
Environment Canada - Freshwater Institute
501 University Crescent
Winnipeg, Manitoba

Second Annual Aquatic Toxicity Workshop (1975)
Dr. G.R. Craig
Ontario Ministry of Environment
Water Resources Branch - Limnology and Toxicology Section
P.O. Box 213
Rexdale, Ontario

Third Annual Aquatic Toxicity Workshop (1976)
Dr. E. Pessah
Environmental Protection Service
5151 George Street
Halifax, Nova Scotia

The next meeting of the group is scheduled to take place November 7-9, 1978, at the Royal Connaught Hotel, Hamilton, Ontario under the sponsorship of:

Dr. P.V. Hodson (416-637-4559)
Fisheries and Marine Service
Canada Center for Inland Waters
867 Lakeshore Road
P.O. Box 5050
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Fourth Annual Aquatic Toxicity Workshop - Organizing Committee

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Editor's Comments

Proceedings of the meeting are presented here in the form of complete papers or as abstracts only. Some papers appear only as abstracts as the authors wished to publish, or had published, elsewhere in a more formalized journal or for one reason or another, did not wish to submit a manuscript for publication. All papers contained in this report have been reviewed by several referees and in some cases have undergone editorial changes. We did not exercise extensive editorial licence to standardize paper format and style of presentation, hence some differences in organization, graphics, etc. are apparent from paper to paper.

No attempt was made to record questions or discussion of formal papers. This is a legitimate criticism of the proceedings and the organizing committee. It was our intent, however, to encourage informality and open discussion at the meeting and we believed that recording of discussion and formalized presentation of questions detracted from such informality as well as presented an editorial nightmare.

Three workshop discussions were conducted at the meeting where participants broke into small groups which simultaneously tackled a written discussion problem. The problems and recommendations of the groups together with a summary of findings are presented in Appendix I.

It was our impression that such discussion groups added a great deal to the meeting's success. They encouraged a friendly and informal exchange of ideas and exposed participants to a variety of viewpoints from numerous disciplines and experience areas. In addition, they provided a sampling of current opinions on controversial issues and are useful in formulating a "state-of-the-art" position among participants. It is our recommendation that similar discussion sessions be included in future meetings.

John C. Davis
Galen L. Greer
Ian K. Birtwell

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* - speaker

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"Nature with a beautious wall
doth oft close in Pollution"
Shakespeare, 12th Night, Act 1, Scene 1

Of the several stone jetties extending across the Fraser River mud flats, only one, the Iona Island jetty, has an extensive wildlife population of molluscs, rats, crabs and birds. This food chain is supported by the Vancouver city sewage outfall. At the same time, however, heavy metals are present in the Iona Island animal community in amounts greatly exceeding concentrations found in animals from other parts of the mudflats. The question of how this thriving community manages to tolerate its load of heavy metals may be partly answered by the presence of a protective protein, metallothionein, which has been found present in all the animals assayed.

Under experimental conditions, young salmonids, held in enclosures containing 0, 1 and 5 ppb Hg, showed an increased level of Hg adsorbed on metallothionein. Only in the case of the 5 ppb fish was an appreciable amount of Hg also adsorbed on the enzyme peak, isolated simultaneously by Sephadex gel fractionation. Since these fish showed a growth rate which was depressed by 50% from the control and 1 ppb Hb-treated fish, it was postulated that heavy metal poisoning only occurs when there is a spillover of heavy metal from the metallothionein to the enzyme peak, resulting in the replacement of metals which are normally associated with enzymes (e.g. Cu and Zn). In the presence of adequate metallothionein induction, as is presumably the case among the Iona Island animals, there is a protection from heavy metal poisoning.

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FIELD BIOASSAY: A VIABLE TOOL FOR BIOASSAY TESTING

by

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ABSTRACT

Inniss, C.S., G.L. Beggs and J.D. Ellis. 1978. Field bioassay: a viable tool for bioassay testing. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Three types of field bioassay, release-recapture, onshore continuous exposure to receiving waters, and caging methods, are reviewed and evaluated. Field bioassays employing cages are recommended. Problems inherent in the field bioassay apparatus and the criteria for a good cage are discussed. Fish culture cages that have proven successful are examined briefly. A cage design, derived from intensive fish culture practices, that incorporates many of the recommendations from the studies reviewed is described. Two field bioassays employing this cage were conducted in a small artificially-destratified eutrophic lake in southern Ontario. In autumn 1976, 96% of caged rainbow trout were recovered in good condition after 6 weeks. A second trial, to compare *in situ* cage bioassays with release-recapture methods, was conducted in the spring of 1977. After 9 weeks 100% of the caged rainbow trout were accounted for while only 4% of released fish were recaptured. A smaller version of the commercial cage, incorporating a demand feeder, was designed, constructed and tested successfully for 4 weeks in the same lake. Other possible applications of field bioassays are suggested. In light of the current concern over diffuse contaminants, this application is emphasized as a better alternative to laboratory bioaccumulation experiments.

INTRODUCTION

Every meeting of workers in the field of aquatic toxicity produces queries concerning the role of field bioassays. There have been several proposals to develop the field test as a standard bioassay methodology, in particular, for use in the evaluation of an effluent with respect to flavour impairment of fish flesh.

A review of the literature reveals that reported field bioassays lack the detail essential for the development of methodologies. In particular, authors have often neglected to report the difficulties, concentrating only on the successes. The would-be tester, basing his methods on the literature, frequently encounters problems, unreported but obviously inherent in the applied technique. Field bioassays cannot be replicated. Their variability must be offset by intensive monitoring of physical and chemical variables. As a result of these limitations the field bioassay has been an under-utilized technique. Researchers have concentrated on laboratory testing, sacrificing natural conditions for controlled experiments.

Several relatively recent considerations indicate that the development of a field bioassay methodology is warranted; the ecological crises engendered by diffuse contaminants such as PCB's and mercury; the discrepancies between laboratory-derived safe concentrations and field observations; and the logistics of laboratory continuous-flow tests (requiring the transportation and storage of several thousand litres of bioassay samples).

A methodology proposal based upon a critical review of available literature is presented and possible applications of the field bioassay technique are suggested.

Field bioassays may be conveniently subdivided into three categories, release-recapture, onshore continuous exposure to receiving waters and caging studies.

Release-Recapture

Beamish (1974) and Beamish and Harvey (1972) described release-recapture techniques to determine whether the loss of the white sucker (Catostomus commersoni) population in Lumsden Lake was a direct effect of low pH or an indirect effect caused by the reduction of food supply. The authors introduced 107 tagged George Lake white suckers into Lumsden Lake in May and June 1968. During June and July, 23 were recovered in gillnets or trapnets and removed from Lumsden Lake. An additional 12 were trapnetted and returned to the water. During an extensive netting program in 1969, only one of the remaining 84 suckers was recaptured. Because recapture of control fish required considerably less effort, the authors concluded that the disappearance and decreased growth of the suckers introduced into Lumsden Lake did not result from the tagging and handling.

4,000 pink salmon fry (Oncorhynchus gorbuscha) were also released into Lumsden Lake in the spring of 1966 and 1967 (Beamish and Harvey 1972). Extensive fishing during 1966-1971 failed to capture any of these fish.

Yearling brook trout (Salvelinus fontinalis) were planted in five small lakes in the Sudbury area in the spring, 1976 (Powell, 1977). Each lake was netted three times during the summer to monitor growth and assess the relative survival. Of 1200 fish planted in Lohi Lake, returns totalled only three fish. As Lohi Lake has no fishing pressure and predation should be equivalent to the other lakes, it was assumed that the fish died. Mortality was attributed to water chemistry, probably high copper levels.

One of the main difficulties encountered with release-recapture bioassays is illustrated in the preceding studies. Returns of introduced fish are often very low and even minimal returns require intensive labour. For example, Powell (1977) gillnetted Lohi Lake for 141.5 hours with a return of only one fish of 1200 planted. None of the 4000 pink salmon stocked in Lumsden Lake during 1966 and 1967 were recaptured despite extensive netting for the following 6 years (Beamish and Harvey, 1972). These authors have concluded from the low recapture yields that mass mortality of planted fish occurred. However, this conclusion based on circumstantial evidence is, at best, an assumption.

Onshore Continuous Exposure

A second alternative for field bioassays is onshore continuous exposure to receiving waters.

Grande (1964) pumped water from the River Otra into plastic troughs located onshore and containing brown trout (Salmo trutta) yearlings, to investigate the causes of a decline in salmon catch. These studies were implemented after it was determined by livebox studies that river water conditions were lethal. Studies were carried out for periods up to 30 days, using waters receiving wastes from a pulp and paper mill and wallboard factory.

Several studies employing the onshore bioassay technique have been conducted by the Ontario Ministry of the Environment.

To measure mercury uptake, rainbow trout (Salmo gairdneri) yearlings were exposed in 300 gallon polyethylene tanks to waters pumped from the St. Clair River (Boelens, 1971). A similar technique was used by Wells (1972) to evaluate fish flavour, growth and mortality of rainbow trout in water pumped from five locations on the St. Clair River. Onshore test facilities were also used to determine whether two reclaimed lakes near Sudbury, Ontario would support a fishery (Baksi and Richards, 1976). Three species of fish pumpkinseed (Lepomis gibbosus), rainbow trout fingerlings and fathead minnows (Pimephales promelas) were exposed to the lake waters in 270 litre tanks. Acute and chronic tests were conducted.

Onsite continuous exposure bioassays have been used by the Swedish National Environment Protection Board to monitor several industries and wastewater treatment plants (Hasselrot, 1975). Test containers with about 30 fish were supplied continuously with wastewaters, diluted to various

proportions. These types of tests have been used to assess flavour impairment, to measure mercury accumulation in fish, and to provide a final control of treated wastewater before its release into receiving waters.

The onshore continuous exposure bioassay is an effective tool in some circumstances, more closely approximating the natural condition than laboratory continuous flow bioassays. Onshore studies eliminate transportation of the effluent and can incorporate the assimilative capacity of the receiving waters. However, the technique does not include all the variables influencing waste toxicity. Test waters are usually pumped from a point source. Fluctuations in effluent toxicity due to uneven mixing, turbulence, oxygen, and temperature profiles in the natural system, may not occur at the point source. On the other hand, lethal conditions occurring at a point source may not be representative of the situation in the water column. Contributing factors including sediment interactions and influences from other components of the biota are not considered in onshore continuous exposure studies.

In-situ Cage Bioassays

The third field bioassay alternative, in-situ cage studies, eliminates many difficulties associated with release recapture techniques and more closely approximates the natural conditions than onshore experiments.

Field bioassays employing cages located in receiving waters are not a new technique. Studies by Vallin (1935) and Hagman (1936) have been mentioned by Hasselrot (1964) in his brief review of field bioassay studies of the Swedish Environment Protection Board. However, most of the published literature deals with studies done in the last two decades.

To study the histo-pathological effect of Kraft pulp mill wastes, Fujiya (1961) exposed Sparus macrocephalus, in corrals, to waste waters. Exposure times ranged from 12 to 24 hours.

Coho salmon (Oncorhynchus kisutch) were exposed to waters receiving aerial applications of emulsified benzene hexachloride spray (Jackson, 1960). The fish were contained in shallow floating triangular live boxes attached to logbooms in Alberni Inlet, and Sprout Lake, British Columbia. Jackson also employed a large rectangular live box divided into five vertical compartments, one foot in height, the top compartment being located just below the water surface. Small rectangular live boxes of a wood frame construction covered with wire or plastic screen were also employed in studies by Todd and Jackson (1961) and Ziebell et al. (1970).

Rainbow trout yearlings were held in cages in the South Saskatchewan River to assess mercury uptake by fish (Uthe et al. 1973). Cages were of two constructions, fir plywood with stainless steel mesh doors and vents, and plexiglass cylinders with one open louvre end and one end covered with a seine net. Plywood cages with screen openings have also been used to investigate the toxicity of chlorinated municipal wastes to rainbow trout and fathead minnows (Basch 1972).

In the Athabasca River, the effects of methoxychlor residues in fish were studied by retaining yearling rainbow trout in wire mesh cages located in the receiving waters. The investigation followed a single application of this black-fly larvicide. Cages of a similar construction have been used in other studies (Herbert et al. 1965, Schouwenberg and Jackson 1966, McCarraher 1971, Morry and Cole 1977).

A variety of mercury studies by the Swedish National Environmental Protection Board have been previously reviewed by Hasselrot and Gothberg (1974). These studies investigated pathways of mercury transport to fish by exposing caged Atlantic salmon (Salmo salar) fingerlings to mercury-contaminated waters. Cages were constructed of knotless nylon mesh fitted with stay rings of 35 or 45 cm. diameter. Hasselrot (1964) also used the cages to study mortality and flavour tainting of one year old salmon and edible pike (Esox lucius) and perch (Perca fluviatilis) retained in Kraft pulp mill effluents. Fish held in cages of knotless nylon mesh are currently being used to examine the suitability of neutralized and neutralized and fertilized lakes near Sudbury, Ontario (N. Yan, personal communication).

A review of the literature uncovered some of the difficulties encountered in caging bioassays. Caging bioassays have usually been conducted in small live box-type cages (Jackson 1960; Todd and Jackson 1961; Uthe et al. 1973; Lockhart et al. 1977; etc.). Test fish were restricted in movement and would thus be unable to avoid local environmental disturbance such as high surface water temperatures. Small cages may also result in stress of fish due to confinement. Anomalous mortalities in control cages have been reported by several authors (Todd and Jackson, 1961 and Lambton Industrial Society, 1973). In long-term studies, confinement of fish in cages of wire or metal construction (Herbert et al. 1965; Lockhart et al. 1977; etc.) may result in the abrasion of test fish and ultimately in the spread of infections. Fluctuations of water levels have also been cited as a problem when cages are located in shallow waters (Morry and Cole 1977). Mortalities due to any of the extraneous factors mentioned, environmental disturbances, stress due to confinement, diseases, etc. will complicate interpretation of test results. Despite these problems, caging bioassays are to be recommended for field studies provided that the criteria presented below are met.

CAGE DESIGN CRITERIA

Ideally, cages should encompass the entire water column if local environmental stresses such as oxygen depletion or high surface temperatures are not part of the experimental objective. In addition, cages must be of a design suitable for frequent inspection of cage and fish condition. An open construction, allowing free circulation of water, is essential. Materials used in construction must resist corrosion, mesh size should be chosen to permit or exclude drift organisms as required. Fouling must always be minimized either through the use of an antifouling agent and/or siting the cage to ensure adequate water circulation. Soft materials will reduce abrasion of test fish. Cage shape should reflect the requirements

of the test site with respect to flow and effluent discharge patterns.

Cage size and stocking density are two important, related variables. Restrictive cages or high stocking density may result in poor growth, abrasion of fish, and ultimately in fungus infections.

Unattended cages may be subject to physical damage due to storms and vandalism. Careful selection of cage location, combined with frequent inspections may eliminate these difficulties.

Cage design, size and construction are fundamental to successful field studies. Mortality or stress of the fish due to confinement should never occur. Fish culture in cages has a history of success and efficient cage designs have been well documented. Culture studies have reported survival and growth of 95% to 99% of fish over periods as long as five months (Collins 1972, Kilambi et al. 1977). Cages employed in field bioassay experiments should reflect the best information available from caged fish culture studies.

Several different cage designs have been used with good success. Kilambi et al. (1977) raised rainbow trout and channel catfish for 4½ months with survival rates of 99% and 97% respectively. Cages were constructed of plastic-coated wire mesh over pine frames. Polystyrene blocks provided floatation. Harvesting and feeding doors were incorporated but regular inspection might have been difficult.

Boydstan and Hopelain (1977) raised Steelhead trout (Salmo gairdneri) in rectangular cages of 0.64 cm mesh galvanized hardware cloth. Cage design included hand winches for lifting the cages from the water. Both this study and that of Kilambi et al. (1977) employed cages of relatively inflexible material which could cause abrasion of test fish. Collins (1972) cultured rainbow trout in a warmwater lake. Survival rate was 99% but all mortalities were attributable to injury of the caudal penduncle caused by contact of the fish with the side of the cage. Hasselrot (1964) and Hasselrot and Gothberg (1974) recommend cages of knotless nylon net to prevent abrasion.

Methodology Study

The success enjoyed by many cage culture operations led Innis and Ellis to test the feasibility of culture cages for use in bioassay applications. The model loaned for evaluation, an Ewonet (R)* Floating Cage, incorporated several desirable features of successful cages.

The Ewonet (R) Floating Cage is available in three sizes 50 m^3, 100 m^3 and 200 m^3. Nets may be interchanged without removal of the supporting frame or loss of fish. The net is suspended from a rectangular frame of salt water resistant aluminum tubes. A cross-bar supported by a vertical frame serves as support for a feed hopper of 50 kilograms capacity. The feeder is solid-state controlled, nickel cadmium powered and programmable. Food is dispensed by a vibrator and the quantity can be controlled by adjustment of dispenser gap. Food loss during hours of darkness is prevented by a photocell. The netting is a knotless nylon with a mesh size of 1.59 mm.

* Supplier -Aquafarms Canada Ltd., R.R. #1, Faversham, Ontario. NOC ICO

The net is suspended from the frame by corner rope loops and rubber straps. Fish may be harvested or inspected by means of a rope attached to the bottom of the net and running over the crossbar. A mesh cover is supplied to eliminate escape and predation of the fish. The mesh is impregnated with a copper-based anti fouling agent.

The cage is easily assembled on site. Six inflatable, polyvinyl chloride floats provide 600 lb. of buoyancy and the assembled unit may be towed into position by row boat. Anchors in the net-pockets and attached to the frame hold the net in position.

The Ewonet(R) Floating Cage has been used successfully by Inniss and Ellis, to investigate the survival of rainbow trout in a small, eutrophic kettle lake, Tory Lake, in southern Ontario. The hypolimnion of the lake was characterized by excessive ammonia levels of about 25.8 mg/l and anaerobic conditions. Artificial destratification with compressed air re-established dissolved oxygen levels and oxidized the ammonia to levels less than 0.5 mg/l. Two caging bioassays were instituted following an unsuccessful stocking of the lake with 300 rainbow trout in May 1976. In the first experiment, 25 rainbow trout were placed in the 100 m^3 cage in the fall of 1976. Six weeks later, 24 trout (one fish was lost in recovery) were recovered from the net and appeared to be in good condition. A subsequent experiment in the spring of 1977 involved the release of 175 trout into the lake and 145 trout into the cage. After nine weeks no mortalities of caged trout were observed despite a heavy cover of Lemna minor (duckweed) and a blanket of Cladophora on the mesh sides. In contrast, only 7 of 175 planted fish were captured in one week of trapnetting. During capture, trapnetted fish suffered abrasions which may later have contributed to mortality. Caged fish were in better condition on removal than at introduction.

The Ewonet(R) Floating Cage, intended for the production of 4-5 tons of marketable fish per annum, is too large and cumbersome for bioassay experiments using small numbers of fish. It could be used to advantage for long-term bioaccumulation or flavour evaluation exposures, requiring periodic harvesting of fish. For other bioassay applications, the authors suggest several modifications. Circular or triangular frames might be less subject to drag and be self-cleaning for river applications. Nets of different sizes which fit a modular, jointed frame would be advantageous. Net depth and shape could then be changed depending on site and experimental requirements. A tapered net attached to a pulley-suspended harvesting rope would facilitate inspection by a single observer. Mesh size could be selected to admit or exclude prey organisms as desired. The use of copper-based anti-fouling agents should be avoided for field bioassay. The substitution of a demand feeder triggered by the fish would increase food utilization and could be employed to test fish response impairment. Automatic samplers and field monitoring equipment, attached to floating platforms, could provide the chemical and physical data necessary to interpret results.

The authors have constructed a smaller net cage (Fig. 1) incorporating some of the suggested modifications. The net is small enough to

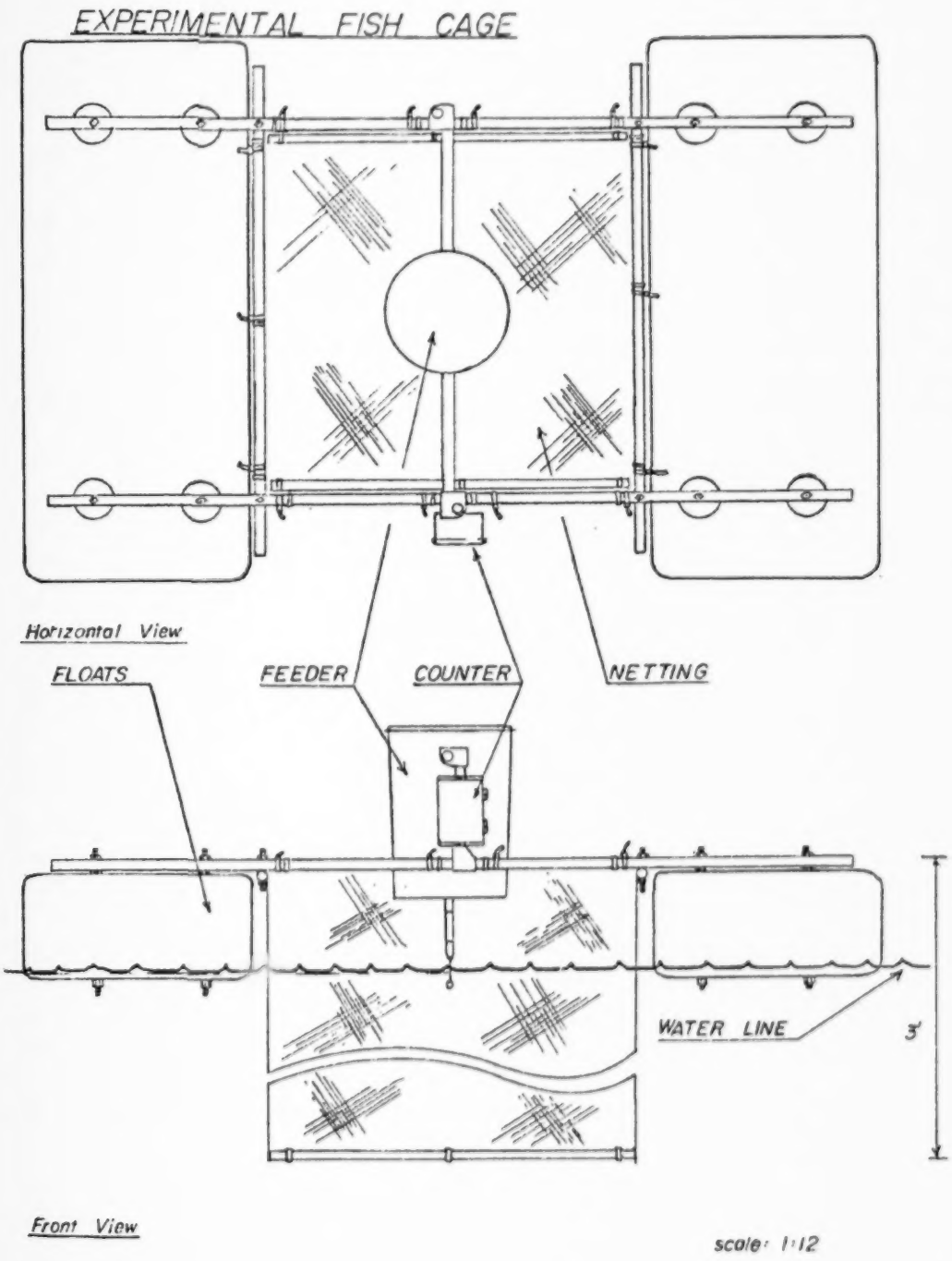

Fig. 1. Line drawing of prototype fish cage.

be transported to the test site by one person. Styrofoam blocks have been substituted for the vinyl floats which are prone to accidental deflation. The cage consists of an aluminum tube frame supporting a nylon mesh net which is readily interchangeable for various water depths. A nylon net cover prevents escape of the fish and predation by birds. Fish may be harvested by raising the bottom of the net by means of a rope passing over the cross-bar which also supports the demand feeder.

The demand feeder consists of a 20 litre polyethylene bucket into which a large plastic funnel has been welded. Food is dispensed when the delivery rod (Fig. 2) is shaken by fish striking the lure. The rod incorporates a switch which makes contact when the rod tube is deformed. A small, hand-held, liquid crystal display calculator serves as a counter, the switch closures being registered in the display and the memory of the calculator. In the prototype, water-proofing of the calculator was accomplished by enclosing it in a plastic bag, in the bucket. Future models will employ a water-proof case as shown in Fig. 2. The silver-oxide batteries powering the calculator will last for several thousand hours provided that air temperatures remain between 0°C and 40°C.

Total cost of the cage was slightly over $200.00. Approximately ten man days were needed to construct and debug the prototype. Later models would require far less labour.

Trials of the feeder in the laboratory indicated that yearling rainbow trout will learn to feed themselves in less than one day especially when food release is initially triggered by hand. A tank of two hundred yearling fish characteristically produced several hundred strikes per day.

Field trials, again conducted in Tory Lake, were successful despite below freezing temperatures which rapidly reduced feeding responses and the efficiency of the calculator batteries. Thirty yearling rainbow trout were held in the cage and all were recovered after 4 weeks. The fish appeared to be in good condition and mean weight and fork length increased over the experimental period.

Various refinements of this cage design have been proposed for later models which will be used for toxicity evaluations in remote areas of Ontario.

Applications of Caging Bioassays

There are numerous applications of the field bioassay technique. Most important, in conjunction with laboratory tests, caging studies accompanied by physical and chemical measurements may provide a reliable indication of the effects of contaminants on aquatic organisms in a near-natural situation. The *in situ* bioassay incorporates the chemical, physical and biological properties of the receiving waters. Such factors alone, or in combination can influence the toxicity of a contaminant. For example, healthy fish populations have been observed in waters with a total copper concentration exceeding reported lethal laboratory levels; it was later determined that the copper was bound to humic acids in the water, effectively limiting its availability to fish (Grande 1966; Zitko 1973).

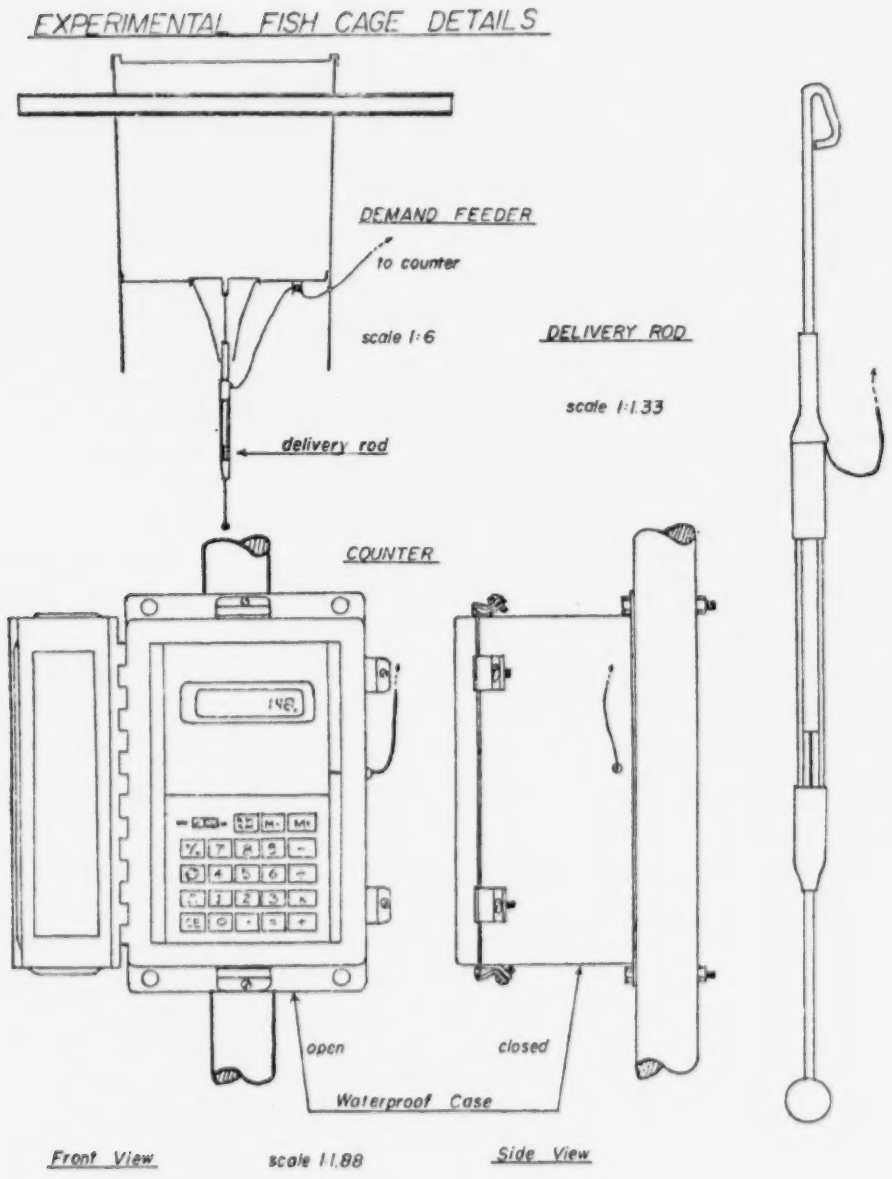

Fig. 2: Line drawing of demand feeder and counter.

The field study using a cage can incorporate toxicant fluctuations and the assimilative capacity of the receiving waters. By combining water sampling for chemical analysis with *in situ* fish cage bioassays at several depths and distances from a point discharge, the mixing zones of waste plumes can be mapped. This combination of chemical and biological information is vital to effluent control programs.

Caging bioassay can be used for both short-term acute studies and a variety of long-term studies. Bioaccumulation studies and the evaluation of flavour impairment are easily conducted in the field. Comparable laboratory continuous flow bioassays for flavour impairment require 3500 litres of bioassay sample to run a 48-hour test with six concentrations and 24 rainbow trout. Other possible long-term sublethal tests include monitoring physiological and histological changes resulting from exposure to a contaminant. Sublethal studies could also utilize the demand feeder, triggered by the fish, to test response impairment of fish exposed to a toxicant. Preliminary trials using caged fish would prevent large scale losses of fish to be stocked in waters of unsuitable quality.

The Swedish National Environmental Protection Board has used field bioassays to continuously monitor receiving waters for changes in effluent quality (Hasselrot 1975) and to detect sources of pollution (Hasselrot 1964).

In light of the current concern over diffuse contaminants the application of the *in situ* cage bioassay in this area cannot be over-emphasized. For example, by conducting fish caging experiments in mercury contaminated waters it may be possible to assess the relative contributions to mercury intake of pathways over the gills or through the food chain. Many of the current theories on mercury pathways have been derived from laboratory experiments demonstrating uptake and accumulation in unnaturally high mercury concentrations (Olson *et al.* 1973; Wobeser 1975; McKim *et al.* 1976) or by assumptions concerning the causes of increasing mercury levels with increasing size, with piscivorous feeding habits, or with increasing trophic level (Ratkowsky *et al.* 1975). A caging study, by manipulating the food source to test animals and by setting the cages contiguous or not with mercury-laden sediments, may help to resolve the growing controversy.

ACKNOWLEDGEMENTS

Our thanks to Mr. Hans Petersen for the loan of the Ewonet(R) Floating Cage and to John Hayes and Alan Lawson who constructed and tested our prototype cage. We also gratefully acknowledge Ms. C. Rendell, Mrs. M. Barclay and Mrs. J. Kurdel for assistance in preparation of this manuscript and our co-workers in the Toxicity Unit for manuscript review.

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ABSTRACT

Hummel, B.L. and M.R. Speyer*. 1978. In situ bioassays in the mining industry. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

This presentation describes the purposes and techniques of the in situ bioassays conducted by the Noranda Research Centre. These tests permit assessment of lethal and sub-lethal effects of effluents on native organisms under field conditions. Short-term lethality has been determined using various salmonids and native marine species exposed in cages. Heavy metal accumulation and growth rate have been monitored by culturing of oysters and blue mussels on trays or ropes. Growth rate and population abundance of lobsters have been evaluated by tagging and recapture. The relationship of these tests to other survey methods is discussed.

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ABSTRACT

Alexander*, D.G. and R. McV. Clark**. 1978. Selection of phenol as a reference toxicant to detect differences in sensitivity among groups of rainbow trout (Salmo gairdneri). Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Phenol was selected as a reference toxicant from among several chemicals in screening bioassays because phenol consistently detected differences in sensitivity among groups of rainbow trout (*Salmo gairdneri*). The chemicals tested in order of increasing consistency of response were dodecyl sodium sulphate, copper sulphate, sodium azide, sodium pentachlorophenate and phenol. Experiments showed that phenol detected differences in sensitivity between strains of trout and could discern the effects of starvation, temperature stress and pre-exposure to 0.04 mg/l chlorine, but not the effects of three brands of food and high mortality during holding. The sensitivity of rainbow trout to phenol was independent of weight and loading density in the bioassays.

The results suggest that rigorous bioassays require that starvation and crowding of fish should not occur in holding facilities and that fish should not be subject to sudden temperature changes or exposure to chlorine in holding facilities and bioassays. No conclusion could be made about the effect of disease and high mortality during holding on the sensitivity of trout to phenol, but in the authors opinion diseased fish should not be used in bioassays.

The use of phenol as a reference toxicant for the rapid detection of differences in sensitivity among groups of fish is limited because differences can only be detected by comparing the sensitivity of an unknown group of fish to that of a known, unstressed group of fish in the same bioassay. The concept of a single reference toxicant appropriate for bioassays with a variety of chemicals is questionable because differences among groups of fish which are detectable by a reference toxicant may not affect the results of bioassays with other chemicals. A series of physiological and behavioural screening tests and diagnostic health checks may be more useful than reference toxicants to identify groups of fish which should not be used in bioassays.

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ABSTRACT

Richards*, Norman L. 1978. Effects of Chemical Use in Offshore Well-drilling Operations[1]. Proc. Fourth Annual Toxicity Workshop, Vancouver, B.C. November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Author summary and discussion

Data on the effects of chemical use in offshore well-drilling operations are very limited. Policy decisions are currently based on static, 96-hr LC_{50} determinations, observations of divers, and theoretical models of pollutant dispersion. A limited research program on drilling-fluid constituents has been initiated at the Gulf Breeze Laboratory to provide a better data base as one component in the evaluation of the relative hazard of using alternative drilling mud constituents and to develop better laboratory methods for xenobiotic evaluation. It is obvious that these methods should include: effects on the structure and function of communities, indirect effects of pollutants, bio-accumulation potential, toxicity of mixtures to organisms indigenous to lease areas, attraction of marine species to chemicals used in well-drilling operations, and mechanism of action of toxicants. In this way, drilling operations can be based on better toxicological information. Hard data on effects might also eliminate unjustified concern about effects of certain chemicals, which, in fact, may be safely used in well-drilling operations.

*U.S. Environmental Protection Agency, Gulf Breeze, Florida

[1]C. Hall and W. Preston. Program Review Proceedings of Environmental Effects of Energy Related Activities on Marine/Estuarine Ecosystems. Interagency Energy-Environment Research and Development Program Report. E.P.A. - 600/7-77-111, October, 1977. pp. 161-173.

DAPHNIA FOR SUPERIOR SUBLETHAL TESTING

by

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ABSTRACT

Westlake, G.F., D.W. Rowe, J.B. Sprague, T.A. Heming, and I.T. Brown. 1978. Daphnie for superior sublethal testing. Proc. Fourth Annual Aquatic Toxicity Workshop. Vancouver, B.C., Nov. 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

A two-year project was conducted to develop rapid and meaningful sublethal toxicity tests and to document the sublethal toxicity of a liquid oil refinery effluent that met current federal regulations. Effluent from a refinery had not previously been examined for sublethal effects. Tests with Daphnia pulex were the easiest and most sensitive in the series of sublethal experiments.

The EC50 for reproduction of Daphnia was 3.1% effluent, with a "safe" EC5 of 0.5%. The test took two weeks, required simple equipment and small samples, and was fairly easy. A 48-hour lethal test with Daphnia was even easier, and predicted sublethal effects on both Daphnia and fish.

Sublethal thresholds for fish were higher and testing was more arduous. Thresholds of about 10% effluent were determined for growth of trout and reproduction of flagfish. The growth test required 1.5 months and had problems such as aggression between fish, while the flagfish test required 3.5 months of intensive work. Trout showed no obvious avoidance of effluent. Locomotor activity increased significantly between 1 and 10% effluent, coughing above 25%, and respiratory rate above 50%. All three tests were relatively complex in equipment, technique, or analysis.

For this effluent, tests with Daphnia were advantageous for comparative research or monitoring. An exception would be for continuous or periodic on-line monitoring, for which computerized assessment of fish coughing would be useful. Some other possible tests were not evaluated because of uncertain ecological significance.

INTRODUCTION

This paper summarizes the main results of a two-year laboratory study on the effects on aquatic animals, of a treated petroleum refinery effluent. There were two objectives in the study: (1) to delineate a relatively rapid and meaningful test of sublethal effect on aquatic organisms for a treated liquid effluent from a petroleum refinery; and (2) to discover whether sublethal effects of such a treated effluent were unusually severe or unusually mild, in relation to acute lethality. There had been no published work on sublethal effects of refinery effluent on aquatic animals (Côté, 1976).

Five kinds of experiments were selected, in order to give a wide assessment of sublethal toxicity. Four of these were with fish and one with Daphnia. The fish tests are covered briefly first for the purpose of comparison. Methods and results are only outlined here; complete details will be available in a forthcoming background report.

EFFLUENT

Experiments were to use effluent that met current Canadian regulations and guidelines (E.P.S., 1974). The effluent was delivered twice a week from a single refinery that used physico-chemical and secondary biological treatment, equivalent to best practicable treatment.

The effluent was of reasonable chemical strength for the experiments, since it approached government guidelines but did not exceed them greatly. The 68 batches used in sublethal experiments consistently met government regulations for pH, phenolics, and sulphides. Averages for oil and grease and ammonia were, broadly speaking, about at the government limit in 3 of the 5 experiments, and a little high in the others. Suspended solids were higher than the regulatory limit because the effluent was collected before final settling.

Non-lethality of the effluent to rainbow trout is specified in federal guidelines. Of 68 batches used sublethally, 60 were judged to have passed the government's 4-day lethal test. Overall, the 68 batches caused a corrected mortality of 11% of the trout, at full strength. This was extrapolated to an hypothetical LC50 of 140% effluent (hypothetical LC50's from 100% to 190% effluent in the various experiments). Thus the effluent was satisfactory for our sublethal experiments since, overall, it met the guidelines for non-lethality, but still came reasonably close to having a lethal effect.

A batch of effluent was not used in sublethal experiments if it caused more than 50% mortality of rainbow trout during the first 24 hours of a lethal test. This criterion resulted in rejection of one batch of effluent for sublethal work, and one-half dilution of another batch before using it. The decision was made at 24 hours in order to avoid undue aging of the effluent before using it in experiments.

GROWTH OF RAINBOW TROUT

Two growth experiments were done, one at excess and one at high feeding rate. Each lasted 44 days, with fish sampled for size every 11 days. Tests used four concentrations in duplicate, in doughnut-shaped tanks with paddle-wheels. Results were very similar in the two experiments, for length, wet and dry weights. Effluent at 30% resulted in severe effects; no significant growth and fin erosion. Effluent at 10% marginal effects - lower weight at 44 days, barely significant compared to the controls, with no effects at shorter times. Concentrations of 3% or lower had no significant effect. It was concluded that the threshold for sublethal effect on growth was in the vicinity of 10% refinery effluent.

The growth experiment had the desirable feature of yielding ecologically meaningful results. Disadvantages of the experiment were: (a) it took 1.5 months; (b) was fairly labour-intensive; and (c) there were problems of aggression between certain sizes of trout, resulting in false starts for both trials.

ONE-GENERATION EXPOSURE OF FLAGFISH

This type of experiment will be familiar to most people (U.S.E.P.A., no date). Briefly, exposure started with one-week-old fish and continued until fish of the second generation were one month old. Seventeen measurements were made at various stages, of mortality, growth, reproductive performance, and abnormalities. Again in this experiment 30% effluent was associated with severe effects. There were significant deleterious effects compared to the control, in 8 of the 17 measured items; for example growth and maturation of the first generation, and abnormalities of the second generation. Effluent at 1% had no significant effect. Exposure at 10% resulted in only one difference in the 17 items - the size of first-generation males at the end of the experiment. Since this apparently did not affect performance of the males, it was concluded that the threshold for sublethal effect on flagfish was again in the vicinity of 10% treated refinery effluent.

The flagfish experiment had the advantage of providing results that would be meaningful in nature, and was considered a searching scan of sublethal toxicity. There were few problems but the experiment did require 3.5 months of intensive effort, including feeding five times every day.

AVOIDANCE AND LOCOMOTORY ACTIVITY

A study of avoidance was considered essential since concentrations causing behavioural response may be quite different from those causing physiological damage. Experiments were carried out in a rectangular tank, with experimental and control streams of water flowing side-by-side through the chamber (Fig. 1). Forty rainbow trout were tested, each at a single concentration of effluent. First there was a one-hour control period with only clean water, than a one-hour experimental period. Throughout each period, position of the fish was recorded with respect to 16 square regions of the tank-bottom. Any measure of performance was expressed as:(performance during control period) minus (performance of the same fish during experimental period).

No obvious avoidance or preference of effluent up to 30% concentration was shown in this experiment. However, there was a generalized increase in locomotor activity at 10% effluent compared to the control period, and a decrease at 30%. A threshold for changed activity of trout was between 1 and 10% effluent.

Such an experiment is highly desirable as a first approach to possible behavioural changes of fish exposed to the effluent under field conditions. However, there are a number of procedural difficulties. It is labour-intensive, requires many trials to allow for variability of fish, and a considerable amount of analysis by computer. It is also difficult to relate the results from a sharp gradient in small containers to potential responses to gradual gradients likely to be found in nature.

COUGHING AND GILL IRRIGATION RATE

The respiration experiments placed more emphasis on discovering a relatively rapid sublethal test, and less emphasis on ecological significance of the findings. Rainbow trout, one per polyethylene chamber, were exposed to single concentrations of effluent. Electrodes in each chamber allowed respiratory movements to be traced on a strip-chart. "Coughs" could be distinguished on the tracing, from normal respiratory movements of the gills. Results for gill irrigation indicated a linear response, i.e. any concentration of effluent increased the rate compared to the control. However, within the design of our experiment, 100% effluent was the only one causing a statistically significant increase. Thus the threshold was between 50 and 100% effluent. Coughing also increased with concentration, showing a threshold between 25 and 50% effluent.

A problem with this experiment was relative insensitivity, at least for the experimental design used. Rainbow trout moved within the chambers, frequently obscuring respiratory records. A more quiescent fish would be better, perhaps sunfish or flagfish (U.S.E.P.A., 1977). The experiment had the benefit of being fast, providing sublethal assessment the same day. Automated measurement of respiration or coughing could be designed for continuous or periodic monitoring of an effluent.

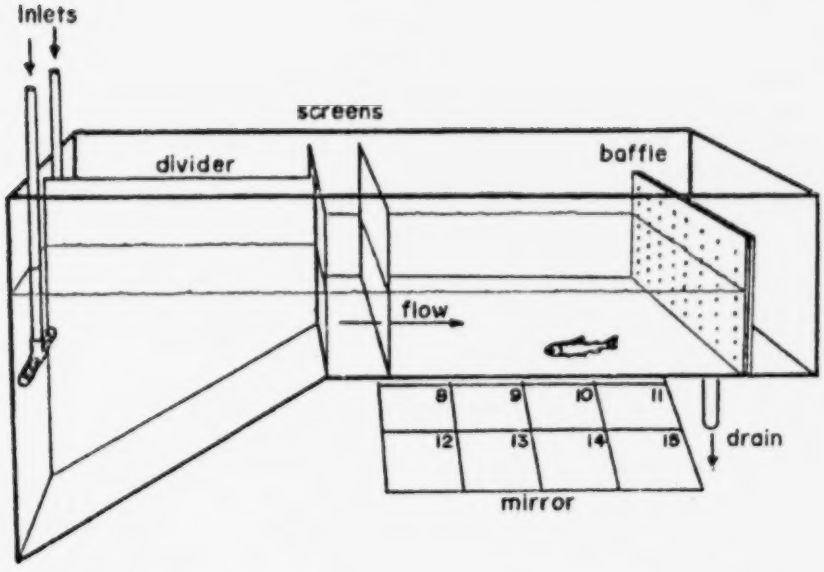

Fig. 1. The tank used in experiments on locomotory behaviour.

TESTS WITH DAPHNIA

Apparatus was the same for both lethal and reproductive experiments. Each chamber of a series contained six ice-cube cups with net bottoms (Fig. 2). A given concentration of effluent in each chamber was renewed twice a day by three successive partial drainings and refillings. A single Daphnia pulex of age two days or less was placed in each ice-cube cup at the start of a test. Mortality and reproductive success was observed using a microscope. Daphnia were fed green alga and finely-ground trout chow, dispersed in each renewal of test-water.

The 48-hour LC50 was 76% effluent. This appeared to be nearly as good a criterion of initial mortality as the 72-hour value. The 24-hour LC50 had a lower slope and higher value, and was considered less satisfactory (Fig. 3). The 10-day and 14-day LC50's were 6.4%, indicating a threshold of chronic lethality. The same batches of effluent appeared to have a hypothetical 4-day LC50 for trout in the vicinity of 190% effluent, as extrapolated from less-than-median mortality. Thus the Daphnia were approximately 2.5 times more sensitive than trout in their lethal response. "Floating" Daphnia were counted as alive since they frequently recovered and reproduced.

Reproduction was satisfactorily assessed by whether or not living young were produced in 14 days. Being an all-or-none response, the 14-day EC50 for reproductive failure could be estimated as 3.1% (Fig. 4). It was decided that reproductive failure among 5% of the Daphnia could be considered as a "safe" level, and this EC5 was 0.52% effluent.

There were not many problems with this test. Traces of chlorine apparently passed the carbon filter and caused control mortality. A few days of aging the dilution-water, aerating it and growing algae in it, solved this. The Daphnia test is not as fast as the coughing/respiratory response. Still it was fast - a sensitive lethal response was obtained in two days, and a chronic study took only two weeks. If replacement of solutions were not required, the apparatus could be considerably simplified.

DISCUSSION

The 48-hour lethal test with Daphnia was judged to be a superior method for screening effluents from other refineries, or for periodic assessment in a given refinery. It had the advantages listed below.

(1) Meaningful. Mortality of test-organisms is irrefutable evidence of toxicity.

(2) Sensitive. The Daphnia lethal test was about 2.5 times as sensitive as that with trout. This means that a valid estimate of LC50 could be made, even if the effluent were well under half of the toxicity allowed by Canadian guidelines. The 48-hour LC50 with Daphnia was not as sensitive as most of the sublethal tests with fish. However if a more sensitive test was needed for some reason, the 2-week EC50 for reproductive failure in Daphnia

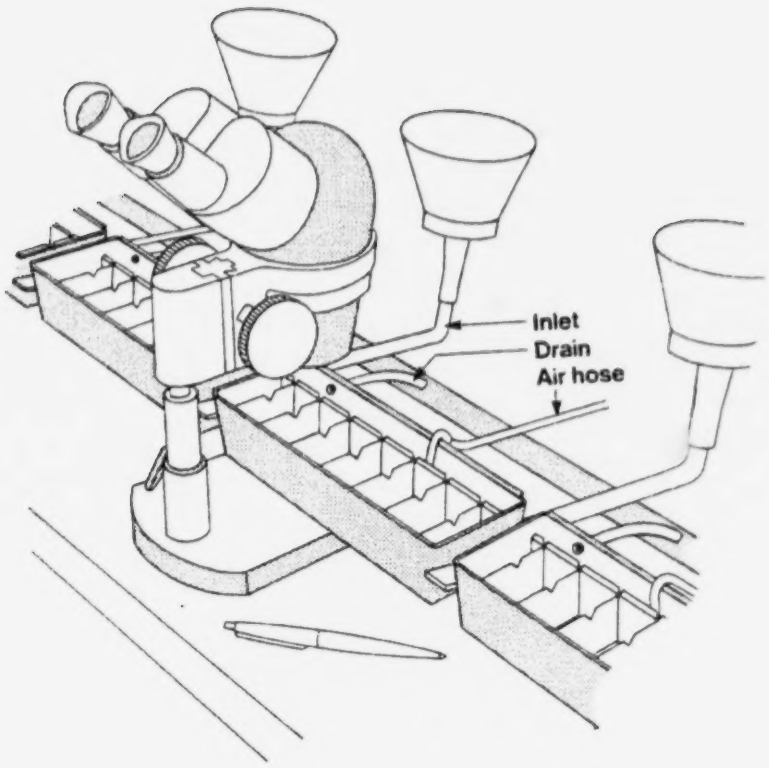

Fig. 2. Sketch showing parts of three chambers, with six cells in each, as used in experiments with Daphnia.

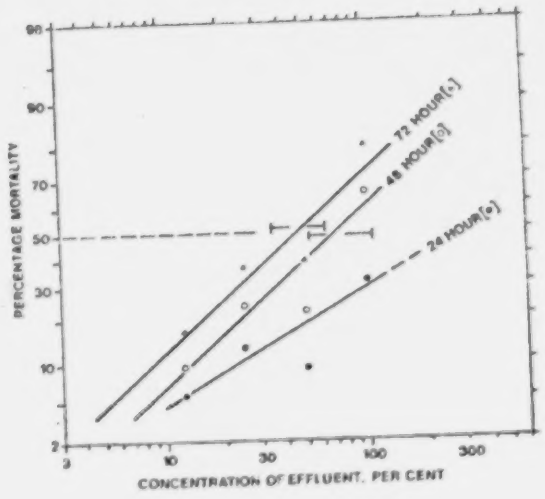

Fig. 3. Acute mortality of Daphnia pulex in treated effluent from a petroleum refinery. Results are for three exposure-times on the same groups of animals. The 95% confidence limits are shown for two LC50's.

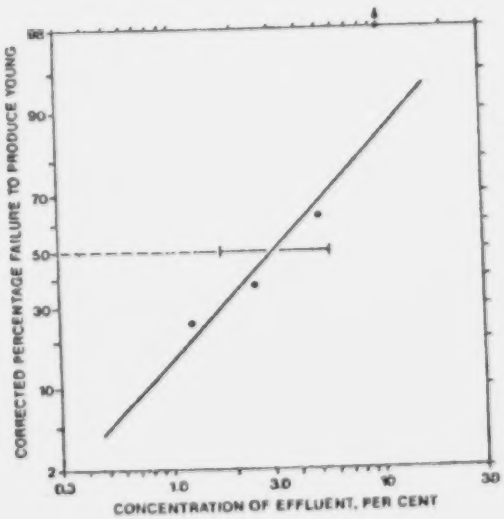

Fig. 4. Reproductive impairment in Daphnia exposed for 14 days to treated liquid effluent from a petroleum refinery.

would be the method of choice for sensitivity and rapidity.

(3) Easy. The test required only simple equipment and procedures.

(4) Small-scale. Only a few litres of effluent were required for tests with Daphnia. This would be a great advantage if tests on a number of refineries were to be done at a central laboratory. The financial advantage would be enormous if samples from northern operations were to be shipped and tested. The test would be particularly useful for pilot studies for which only small quantities of effluent were available.

(5) Fast. The 2-day test-duration is obviously a useful feature. Coughing or respiratory rate of fish would be faster but would require extensive apparatus and analytical capabilities, justified only under conditions of continuous or frequent periodic monitoring.

(6) Predictive. Since the toxic qualities of our refinery effluent were relatively constant during the two years of experiments, it is warrented to estimate application factors from the 48-hour LC50 of Daphnia, to sublethal responses in this and other species.

The 2-day Daphnia LC50 x 0.007 = "safe" level for Daphnia reproduction

The 2-day Daphnia LC50 x 0.13 = "safe" level for fish as judged by trout growth and flagfish reproduction

Other rapid tests are available, such as the useful ones developed by the B.C. Research Council (e.g. Gordon and McLeay, 1977). We were not able to compare these within the period of the contract.

Tests with Daphnia magna were less sensitive than those with fathead minnows, according to one article of a recent newsletter from Duluth (U.S.E.P.A., 1977). However, the comparison seemed to be between chronic lethality in Daphnia, and reproductive success in fathead minnows. Another article in the same newsletter concluded that in tests with a real effluent, Daphnia was more sensitive than the fathead minnow, agreeing with our findings.

ACKNOWLEDGEMENTS

We thank Ken Flood and William Yasui for their work in this project.

This research was initiated by the Petroleum Association for Conservation of the Canadian Environment. PACE provided direct funding in the first year, shared funding in the second year, and ancillary costs. The Canadian government, through the Environmental Protection Service, provided laboratory facilities at the Canada Centre for Inland Waters, shared funding in the second year, and chemical description of the effluent through the Water Quality Branch of the Inland Waters Directorate. We sincerely thank the people in those agencies who helped, so numerous that time available at this conference does not permit naming them.

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USE OF LARVAL HERRING IN BIOASSAYS

by

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ABSTRACT

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Herring larvae are receiving increased attention as a representative of inshore ichthyoplankton for use in marine toxicology. This report includes results of acute lethal tests with bleached kraft mill effluent (BKME) and sodium pentachlorophenate on larval Pacific herring, *Clupea harengus pallasi*. This study is the first reported sublethal developmental bioassay on larval herring, which tested "real life" doses of BKME. A short-term sublethal test is described which evaluated the effects of brief periods of anaesthesia* by BKME on subsequent feeding success of larval herring. A hypothesis is advanced to explain differences in feeding behaviour under various laboratory conditions. Handling procedures, test containers and feeding regimes are discussed.

* Explanatory note: 'anaesthesia' is taken to indicate an anesthetic-like effect - i.e., "immobilization".

INTRODUCTION

Recent literature on the toxicology of aquatic organisms has emphasized the value of using early developmental stages as experimental material (Rosenthal and Alderdice, 1976; Sprague, 1976). In general, embryonic and larval stages appear more sensitive to pollutants than the juvenile and adult stages. Bioassays on these early developmental stages have yielded information useful to the establishment of water quality criteria (Sprague, 1976). In a review of partial and complete life-cycle toxicity tests, McKim (1977) concluded that sublethal tests on embryo-larval or early juvenile stages of fish yield estimates of maximum acceptable toxicant concentrations (MATCs) which are within a factor of two of actual MATCs.

In marine toxicity assessment, developmental stages of the herring, *Clupea harengus*, are receiving considerable attention because of the commercial and ecological importance of the species. Among others, Hjort (1926) and Stevenson (1962) have documented the susceptibility of larval herring to high mortalities. The tremendous fluctuations in herring populations, as well as in populations of other commercially important species, are considered to result largely from these yearly variations in survival (Hjort, 1926; Hempel, 1965). Larval herring are a superabundant and typical component of the ichthyoplankton which can be assumed to share the sensitivity to pollutants of other planktonic larval forms. Thus, utilizing the early developmental stages of herring in partial life history bioassays may provide useful information on sublethal effects *in situ*.

Bioassays have been performed on herring larvae by various investigators. Kinne and Rosenthal (1967) observed the effects of four concentrations of sulfuric pollutants ($FeSO_4$, H_2SO_4) on behaviour and survival of larvae 1 - 6 days old. Linden (1975) made observations on the effects of oil and oil dispersants on behaviour and survival of yolk-sac larvae. Wilson (1972) compared the 100 h LC50's of oil dispersants for embryonic versus larval stages and found that the larval stage was the more sensitive (20 ppm vs 8 ppm, respectively). Wilson (1977) also found that the differences in sensitivity to oil dispersants were less between larvae of six fish species (including herring) than between different developmental stages of larvae of any one species. Struhsaker *et al* (1974) found that larval Pacific herring are more sensitive than embryos to low concentrations of benzene, although the effects on larvae appeared to be reversible. Blaxter (1977) reported that Atlantic herring larvae were more resistant to copper during the yolk-sac stage than during embryonic stages. Alderdice and Velsen (1971) studied the effects of various salinity/temperature combinations on embryonic development and viability of hatched larvae of Pacific herring.

In addition to bioassay literature on herring larvae, various studies have been directed toward mariculture techniques for larval herring (Blaxter 1968; Talbot and Johnson, 1972), feeding behaviour/food

requirements of larval Atlantic herring (Rosenthal and Hempel, 1970), the development of schooling behaviour in Atlantic herring (Rosenthal, 1968), the capacity for avoiding oil dispersants in Atlantic herring larvae (Wilson, 1974), and vertical migration of Atlantic herring larvae under controlled light intensities (Blaxter, 1973). All of this information is applicable to toxicological investigations of herring larvae, especially in sublethal testing.

This paper summarizes results of acute lethal, short-term sublethal and chronic sublethal tests of bleached kraft mill effluent (BKME) toxicity to larvae of Pacific herring, *Clupea harengus pallasi*. As well, the toxicity of sodium pentachlorophenate (NaPCP) to larvae is included for reference purposes. The experiments reported here formed part of a series of acute lethal and chronic sublethal tests of BKME toxicity to the eggs, larvae and juveniles of Pacific herring. The work with larval stages of herring included the first sublethal growth and development bioassay on herring larvae. The results of this study indicated considerable potential for using this economically important fish in marine toxicology. Recommendations for procedures to follow in bioassays with herring larvae are presented.

MATERIALS AND METHODS

ACUTE LETHAL TESTS

Larvae from the first hatch of herring were placed into two series (NaPCP, BKME) of range-finding concentrations, as well as into replicated controls for determination of the maximum residence time which produced only baseline mortalities. The results provided a basis for selecting ranges of toxicant concentrations and for determining the time period used in acute bioassays.

Eggs were incubated in heavily aerated, flowing sea water of 10°C and 24 °/oo salinity. They were hatched in a 1000 l rearing tank, also having flowing sea water, as well as a simulated natural photoperiod. The preliminary tests and bioassays were conducted in a 1000 l tank adjacent to the rearing tank and having an identical seawater source and flow rate so that temperature and lighting conditions remained constant in the transfer from rearing to experimental chamber. A small cup was used to scoop up larvae from the rearing tank, the transfers lasting only a few seconds. All glass bioassay jars were floated in the experimental tank and were held in place by a double mesh of polyethylene lines stretched above and below the water surface. Acute bioassays were also periodically conducted in a constant temperature room (10°C). The water in the bioassay jars was one liter (including effluent). Post-hatch larvae were introduced in numbers greater than or equal to twenty, the actual number being determined post-hoc to minimize handling mortalities. With 51-day larvae only 10-20 were available per jar.

Acute bioassays with both the reference toxicant and BKME were performed with sea water obtained from the Vancouver Public Aquarium. The results of these bioassays were used to determine the relative sensitivity of larval and juvenile forms.

The 96 h LC50's were calculated by fitting a line to log-probit data, expressed as concentration vs percent mortality at 96 hours according to the nomographic procedures of Litchfield and Wilcoxon (1949). Where partial mortality data did not permit calculation, LC50's were estimated graphically from the log-probit (Sprague, 1969).

ACUTE EXPOSURE/RECOVERY OF FEEDING

An acute exposure/feeding recovery bioassay was designed to determine whether recovery from anaesthetic effects of BKME would be complete enough to permit initiation or resumption of normal feeding behaviour. Using four effluent concentrations (0.001, 0.005, 0.01 and 0.05 Toxic Units) plus control, the effect of a 48 h exposure followed by removal of effluent and replacement of fresh sea water, accompanied by introduction of *Artemia* as food, was evaluated using a criterion of "percent successful recovery and initiation (or resumption) of feeding". Solution transfer was achieved by slowly siphoning out 95% of the test solution through a tube protected by 50 micron Nitex meshing, then siphoning in fresh sea water. The bioassay jar was tilted as the test volume was lowered to prevent larvae from being stranded or contacting the siphon meshes. For the two higher effluent concentrations and the control, the solution transfer procedure was performed twice. In addition to the exposure/recovery series, a static exposure bioassay was conducted in parallel (without effluent removal) to evaluate the influence of BKME on initiation of feeding.

CHRONIC SUBLETHAL BIOASSAY

The sublethal bioassay encompassed the early larval stages of herring. Larvae were mass hatched in a 1000 ℓ tank by brief air exposure when embryos were mature. Tanks were stocked with about 500 newly hatched larvae, which were removed from the 1000 ℓ source stock tank with a 200 $m\ell$ glass beaker which was gently inverted below the water surface of the experimental tank. The experimental system utilized polyethylene trash containers, filled to 50 ℓ volume, and modified to suit the needs of planktonic larvae. Each tank had a centralized light source to attract herring larvae and their food organisms away from the tank walls, which were painted flat black for the same purpose.

Larvae were fed to excess daily with newly hatched *Artemia* nauplii. Water temperature was maintained by immersing all of the tubs in a water table with running sea water at 10°C (Alderdice and Velsen, 1971). The

overhead lights were fixed at a distance providing 500 lux at the water surface. Lighting included simulated twilight periods and low level night-time illumination (Marliave, 1977).

The chronic sublethal bioassay encompassed a range of BKME concentrations equal to 0.0005, 0.001, 0.005, 0.010 and 0.050 Toxic Units. Duplicate sets of tanks were used for each of these effluent concentrations along with duplicate controls.

Sanitation requirements dictated 24 hour replacement of part of the medium (arbitrarily set at 10 liters or 20%). When debris was siphoned from tanks, dead and moribund larvae were removed and counted. On days 10, 21, 32 and 42 approximately ten fish were removed from each tank and preserved with 2% formalin in sea water. They were measured to the nearest 0.5 mm and ranked according to gut contents and development stage. Features such as development of various pigment patterns and the initial development of fin rays (caudal, dorsal, anal and pectoral) were noted where possible, and related to total length. Efforts were made to note any behavioural differences, as well as morphological anomalies. In addition, differences between treatments were evaluated by comparing mortality patterns, overall survival and average growth (in length).

RESULTS AND DISCUSSION

ACUTE LETHAL BIOASSAYS

Data from acute lethal bioassays with NaPCP are summarized in Figure 1 comparing larvae vs metamorphosed 180-day juveniles. An increase in sensitivity to NaPCP was observed with age, from newly hatched to 51-day old larvae. A very sharp reduction in tolerance was observed between 0 and 14-day old larvae with the LC50 values generally levelling off at 32 (confidence limits 25, 42) ppb and 17 (range 0, 25) ppb. Comparing these results with 96 h LC50's of 50-70 ppb NaPCP for juvenile rainbow trout and stickleback (E.V.S. Consultants, 1977),it is apparent that herring larvae, after yolk-sac absorption, were about twice as sensitive.

Raw data for acute lethal bioassays with BKME are summarized in Table 1. 96 h LC50's were conducted on 0-day old, 31-day old and 51-day old larvae. Both 0-day old and 51-day old larvae were at least two times more sensitive than juvenile rainbow trout and stickleback to BKME (Table 1). This difference in sensitivity was also observed with NaPCP. However, unlike those tested with NaPCP, 0-day old herring larvae were as sensitive as 51-day old larvae to BKME in acute lethal bioassays.

Figure 1 Acute lethal bioassays with herring larvae and the effect of age on sodium pentachlorophenate toxicity. 0 day and 51 day larvae tested in replicate (salinity 24-27 °/₀₀, temperature 10-11°C). Juvenile herring indicated at 180 days, at a salinity of 20 °/₀₀ and temperature of 15°C.

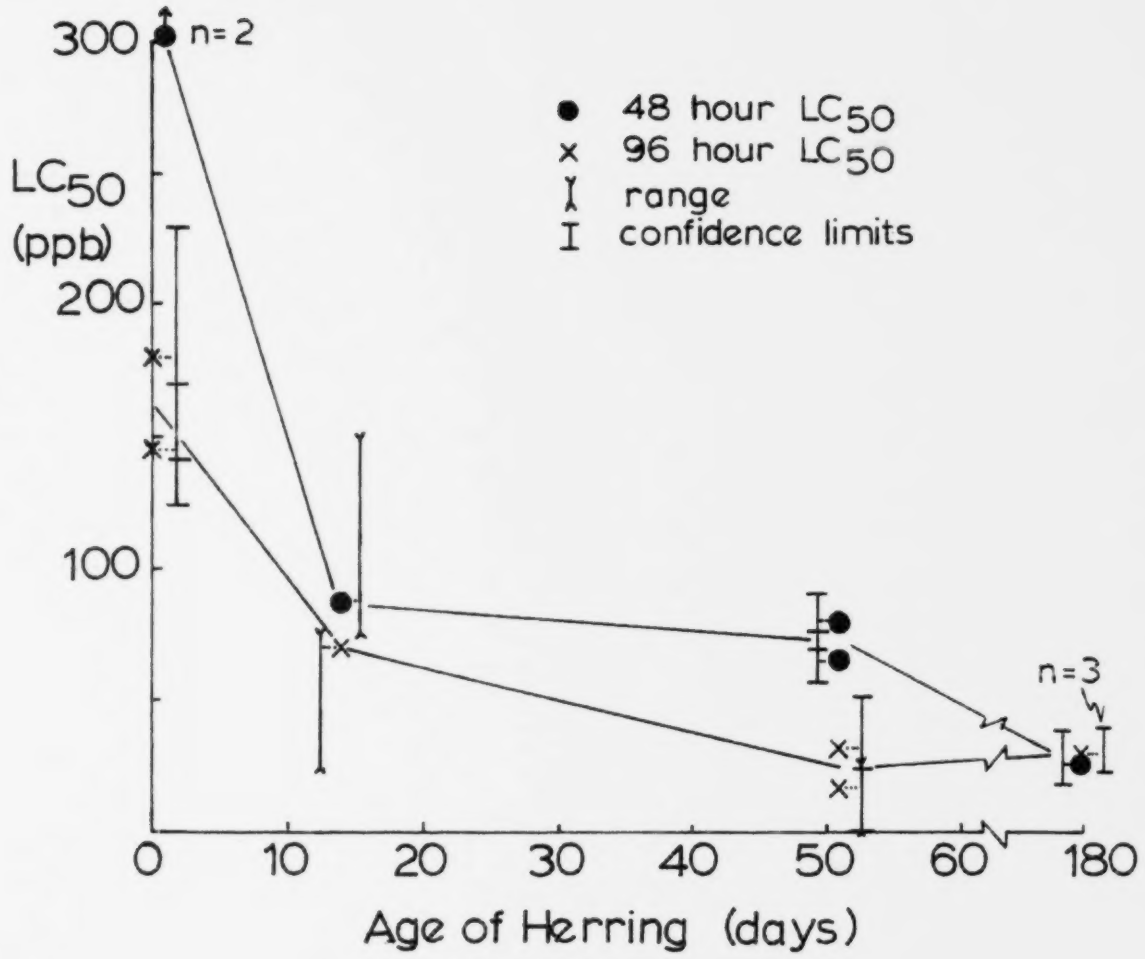


Table 1. Summary of Acute Lethal Bioassays on Stickleback, Rainbow Trout and Herring Larvae with Kraft Pulp Mill Effluent (Confidence limits, c.l. are indicated in brackets, or ranges, r.

Effluent Sample Number	Date Collected	Stickleback			Rainbow Trout			Herring Larvae			
		M.S.T. (h)	96h LC50 (% v/v)	Toxic Units	M.S.T. (h)	96h LC50	Toxic Units	48h LC50 (% v/v)	Toxic Units	96h LC50 (% v/v)	Toxic Units
H1	27-01-77	96	100	1.0	96	100	1.0				
H2	28-01-77	60	82 (c.l. 71.4, 94.2)	1.2	96	100	1.0				
H3	29-01-77	10	35 (r. 18, 56)	2.9	3.8	43 (r. 32, 56)	2.3				
H4	30-01-77	96	100	1.0	11.2	69 (c.l. 62.8, 75.8)	1.5				
H5	18-02-77	96	100	1.0							
H6	24-02-77	70	48	2.1							
H7	04-03-77	60	82	1.2							
H8	01-04-77	96	100	1.0							
H9	15-04-77	96	100	1.0							
H10	03-05-77	14.7	45	2.2	8.5	30	3.3	3.5[b] (c.l. 2.1, 5.9)	28.6	18[a] (c.l. 14, 23)	5.6
								4[b] (r. 2.5, 5.0)	25.0	16[a] (c.l. 13, 19)	6.3
								4.4[b] (c.l. 3.8, 5.0)	22.7		
H11	16-05-77	64.2	84	1.2	15	46	2.2	20[c] (c.l. 13.3, 30.1)	5.0	15[c] (c.l. 11.2, 20.1)	6.7
								22[c] (c.l. 16.0, 30.2)	4.6	15[c] (c.l. 11.9, 18.9)	6.7

a = 0 day old herring larvae
b = 31 day old herring larvae
c = 51 day old herring larvae

ACUTE EXPOSURE/RECOVERY BIOASSAYS WITH HERRING LARVAE

Range-finding bioassays with yolk-sac herring larvae failed to produce lethal effects but indicated that anaesthetic effects increased proportionate to BKME concentration only with an acutely toxic batch of BKME. Anaesthetized larvae from treatment in 0.10 Toxic Units (10% of 96 h LC50 v/v) displayed immediate recovery of orientation and activity when placed in fresh sea water. Immobilization was observed in test concentrations as low as 0.005 Toxic Units (Figure 2).

Since herring larvae could not initiate feeding when anaesthetized, a series of tests was conducted in which percent successful recovery from effluent exposure and initiation of feeding were used as criteria for deleterious effects (Figure 3).

Of the three tests initiated with 0-day and 31-day larvae, no anaesthesia was detected at BKME levels up to 0.05 Toxic Units. Although results varied substantially between tests, the percentage of fish having gut contents was equivalent between treatments in any one test. Most fish with gut contents had only trace amounts of food present, in contrast to the high percentages of fish found to be feeding and the heavy gut loading found in samples from the source stock (62 - 78% feeding, most with guts only half full). There appeared to be no obvious tendency for BKME to prevent initiation of feeding, comparing test treatments with controls. Furthermore, removal of effluent and replacement with clean sea water did not cause increased feeding success.

It is evident from examination of Figure 3 that a difference existed between results of the two tests with yolk-sac larvae. This difference could either be due to differences in the condition of the larvae or in the time allowed for establishment of feeding (4 vs 2 days, for tests with 0 vs 1-day larvae, respectively).

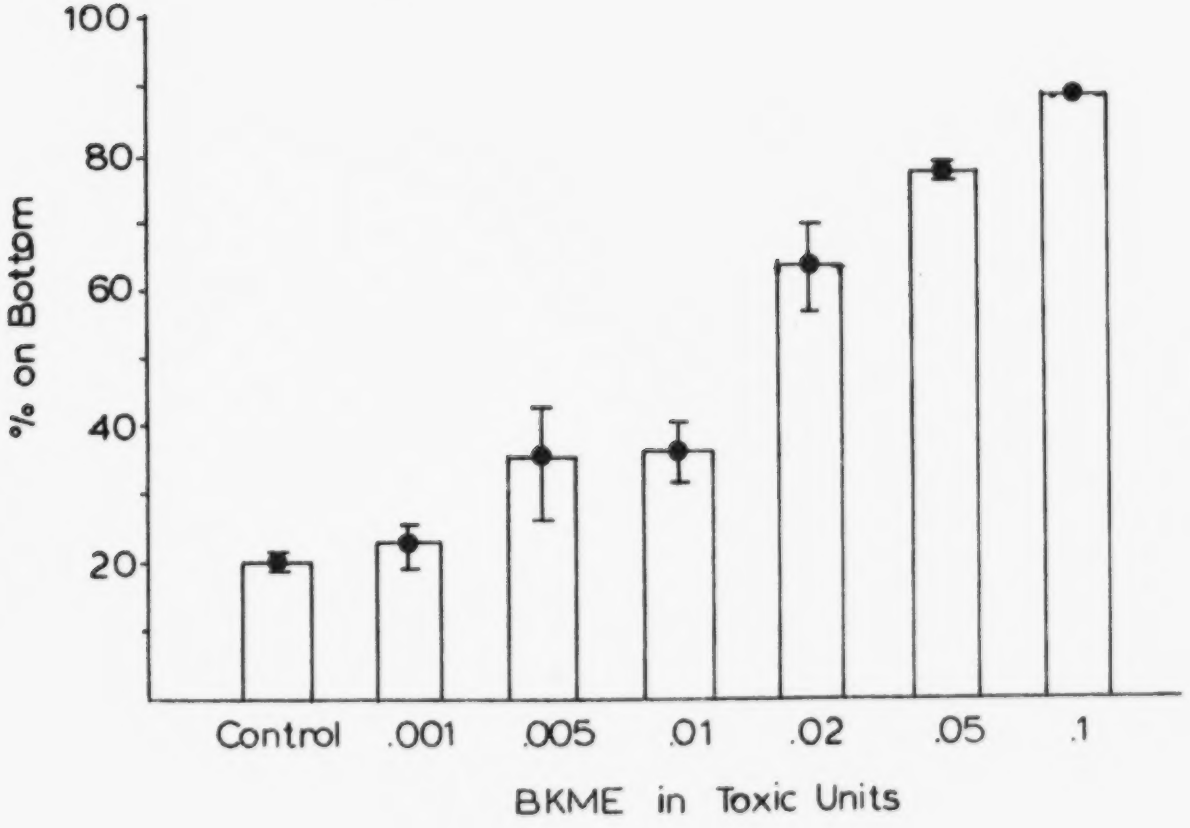
This series of tests was inconclusive, partly because of the non-lethal nature of the specific BKME samples in contrast with the acutely lethal effluent used in the range-finding test, but mainly because of the inability of control larvae to establish high levels of feeding activity in the confines of bioassay jars. Larvae tended to accumulate against the translucent sides and it is possible that the test jars were too small to allow normal feeding behaviour. Further studies using effluent that was acutely lethal, together with larger bioassay containers would provide more definitive information regarding the feeding recovery response.

CHRONIC SUBLETHAL BIOASSAYS

The chronic sublethal bioassays monitored growth, gut contents, development and mortality rates at *in situ* effluent concentrations. Growth, as determined by body length of larvae of four age groups (Figure 4), showed no significant differences* between treatments ($P < 0.05$) based on pooled replicates. Comparing Figure 4 with Figure 5, for each age group sampled, the average length of larval fish was significantly shorter in all sublethal test treatments than the average length of larvae reared in the 1000 l tank. This unde

* Student's t-test.

Figure 2 Anaesthetic Effect of BKME on Herring Larvae after 7 days of Continuous Exposure. (Data points indicate average and range of replicates).



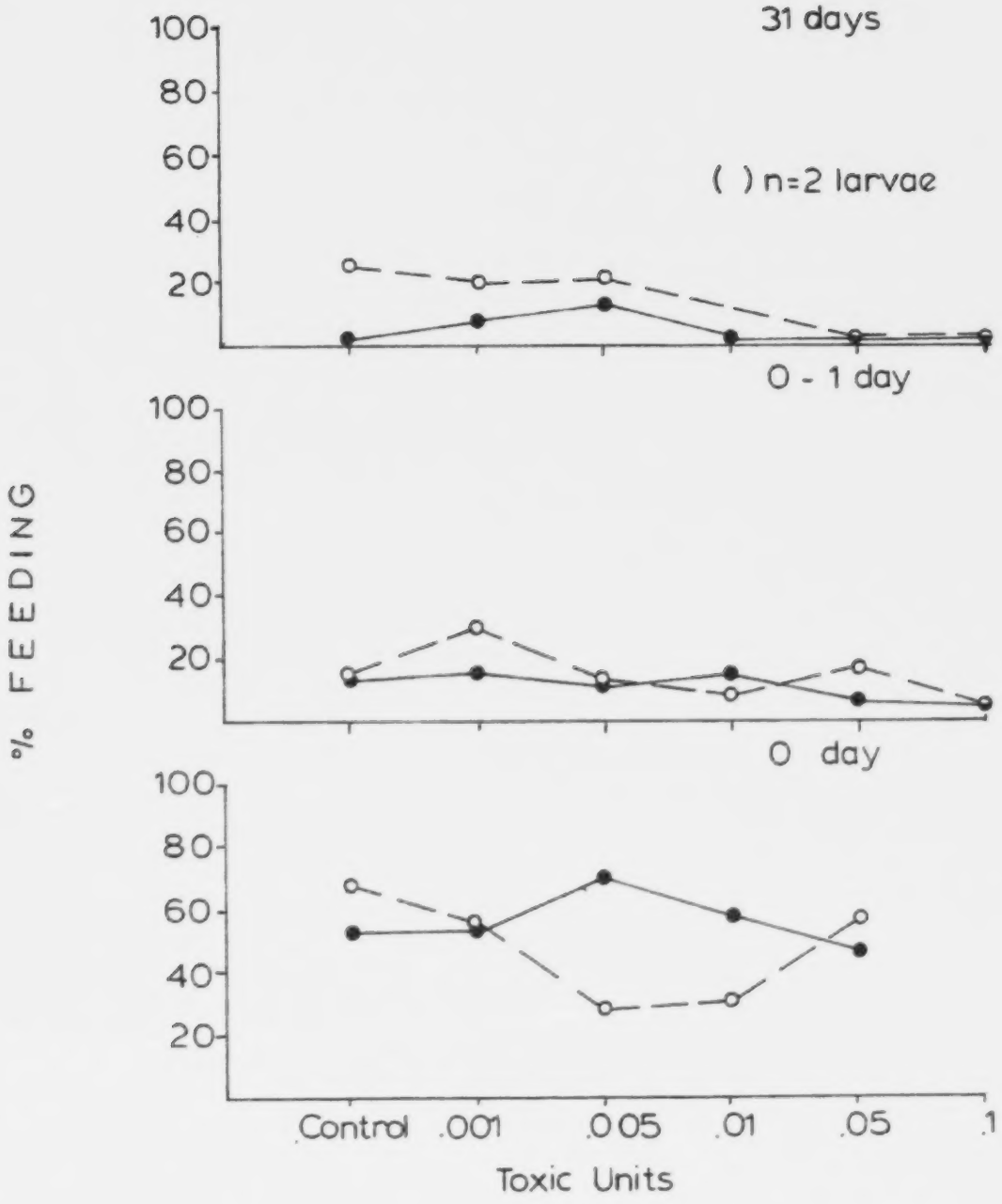


Figure 3 Results of "Short Term Exposure/Recovery of Feeding" tests. Graphs depict percent of surviving larvae having gut contents (dashed line - exposure/recovery, solid-static exposure).

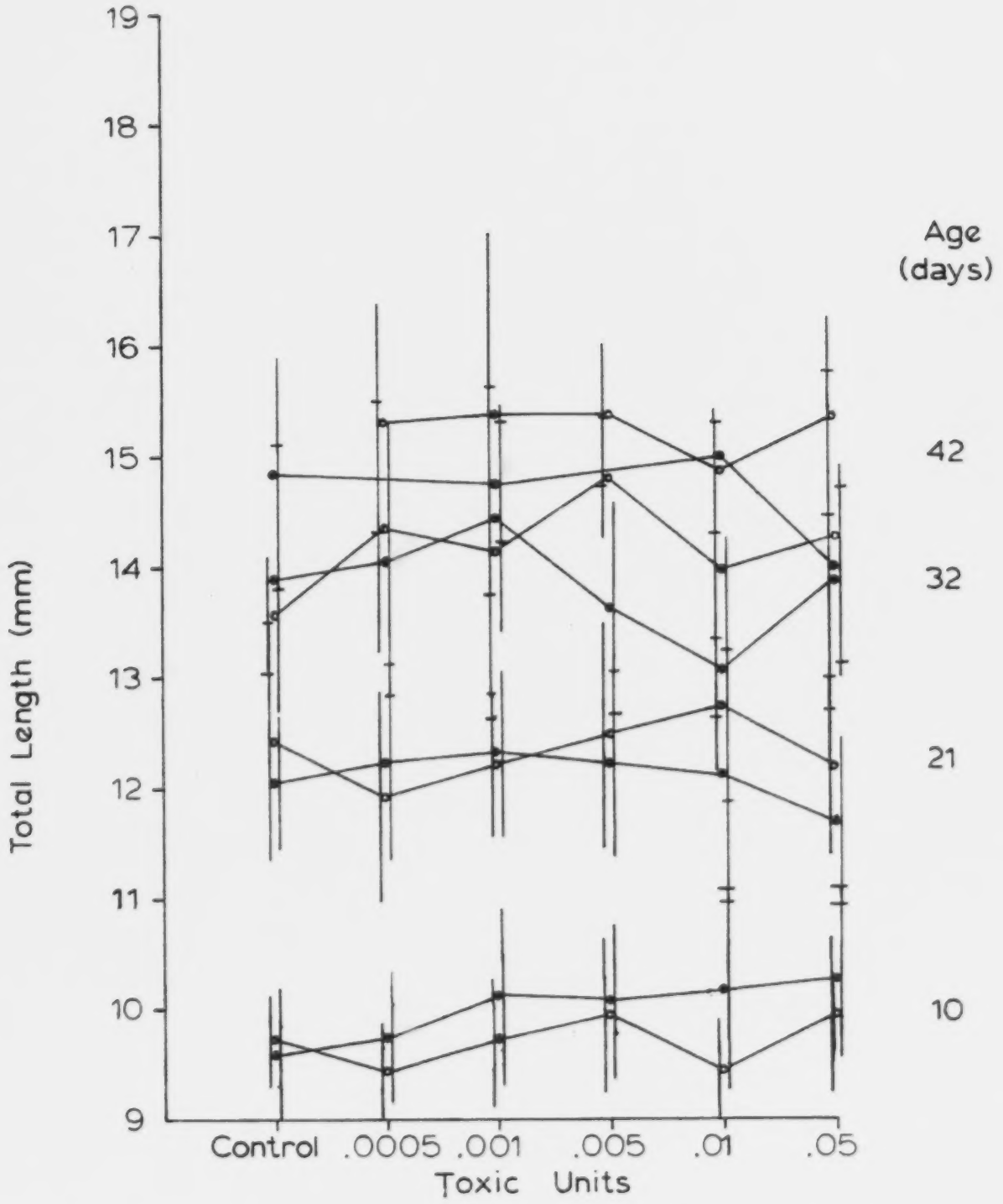


Fig. 4 Average length of herring larvae in samples from sublethal exposure tanks (open and closed circles - replicate tanks). Vertical lines indicate ranges of data.

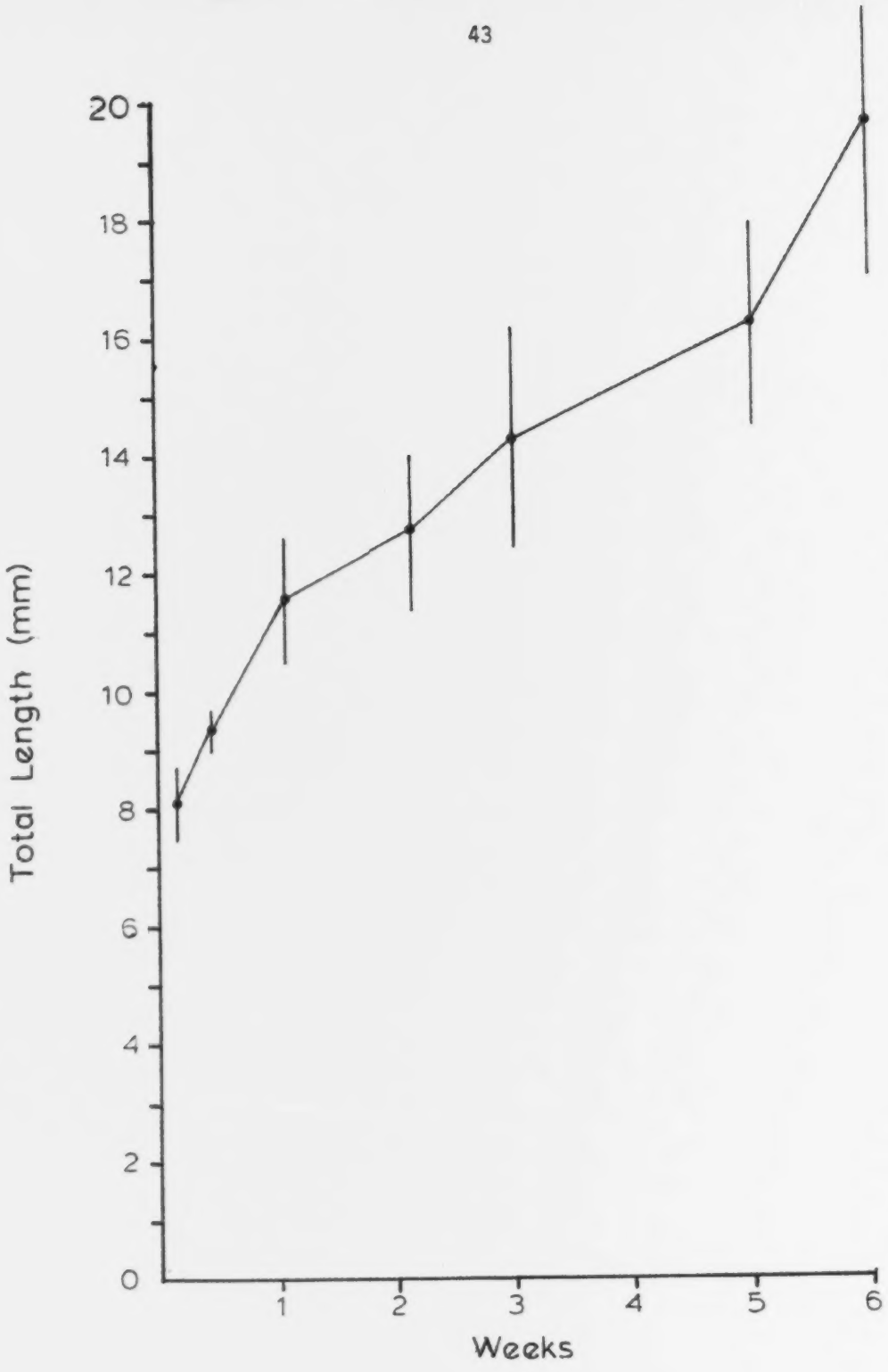


Fig. 5 Average length of herring larvae in samples from 1000-liter tank (source stock for acute lethal tests).

lines the fact that the 50 l rearing chambers, dictated by the logistics of maintaining twelve experimental groups, were suboptimal for laboratory rearing studies. The growth rates achieved in this chronic sublethal experiment were not, however, below the range of growth rates reported in the literature. For example, larvae in this sublethal experiment grew to approximately 14 mm total length in 32 days, as compared to 13 and 14 mm average total length at 35 days reported for larvae in two studies in which herring were successfully reared past metamorphosis (Blaxter, 1968; Talbot and Johnson, 1972). The growth obtained in the stock tank in the present study demonstrated that different growth rates occur under various environmental conditions.

Blaxter (1968) presented data demonstrating the relative nutritional inadequacy of a strict *Artemia* diet in comparison with a wild plankton diet. Blaxter found that larval survival dropped sharply at sizes between 15 and 20 mm total length (none lived beyond 25 mm) when *Artemia* alone was offered, whereas wild plankton supported survival to juvenile sizes (over 40 mm). In contrast, Talbot and Johnson (1972) had successfully reared Pacific herring using *Artemia* nauplii as the sole food source. However, the initial number of herring larvae and larval mortality data were not given. In the present work, feeding behaviour appeared normal and comparable between treatments (Figure 6), using a strict diet of *Artemia*. Gut contents were visually similar across all treatments, including controls. There was, however, an obvious reduction in feeding intensity over the course of the experiment. This feeding reduction also became evident in the stock tank at a later stage (51-day age). The results of this study corroborate Blaxter's(1968) finding that *Artemia* alone will not support survival to metamorphosis. Using techniques comparable to those used for the stock tank, except giving dietary supplements of sieved wild plankton, even more rapid growth and development have been obtained (Marliave, pers. obs.; Rosenthal, 1968).

Development varied according to length. Any variability in developmental stage which occurred at a particular larval length was observed among individuals sampled from any treatment, including controls. The most noticeable abnormalities were variations in jaw length, but again, the full range of these variations also occurred within the controls.

Mortalities in each treatment were summed over one week periods. Mortalities for replicate treatments were also summed and the weekly mortality rates expressed as percentages revealed similar trends in mortality rates between treatments (Figure 7). The average percent daily mortality for summed replicates was 7.8 percent for controls and 7.3, 7.6, 7.6, 8.0 and 7.6 percent for treatments (.0005, .001, .005, .01 and .05 Toxic Units BKME respectively). Total mortalities in the chronic sublethal experiment did not differ significantly between treatments (chi-square test for equality not significant, $X^2 = .0641$).

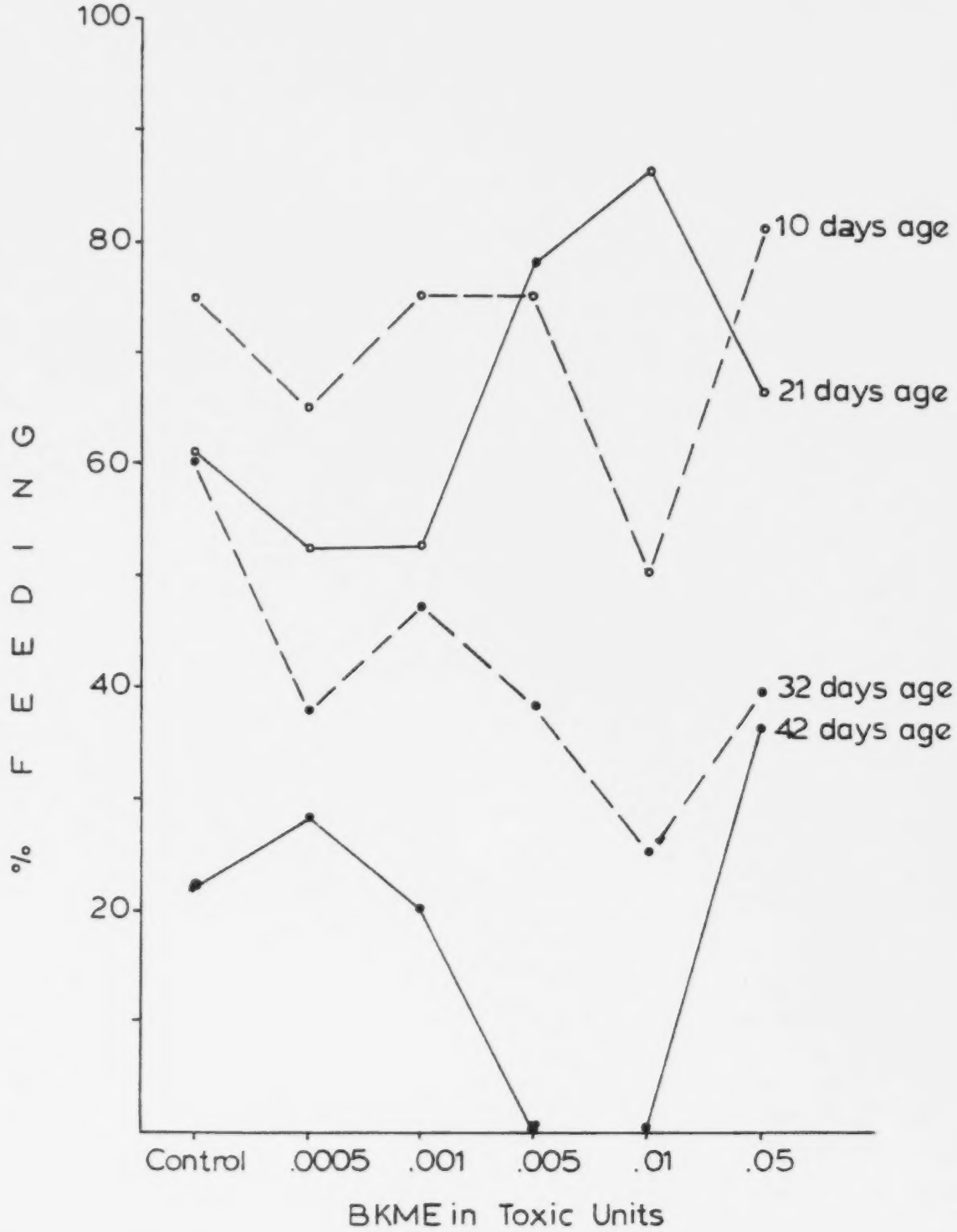


Fig. 6 Percent of larvae feeding at 10, 21, 32 and 42 days of age at various concentrations of BKME stock. (N.B. the data from replicates is pooled).

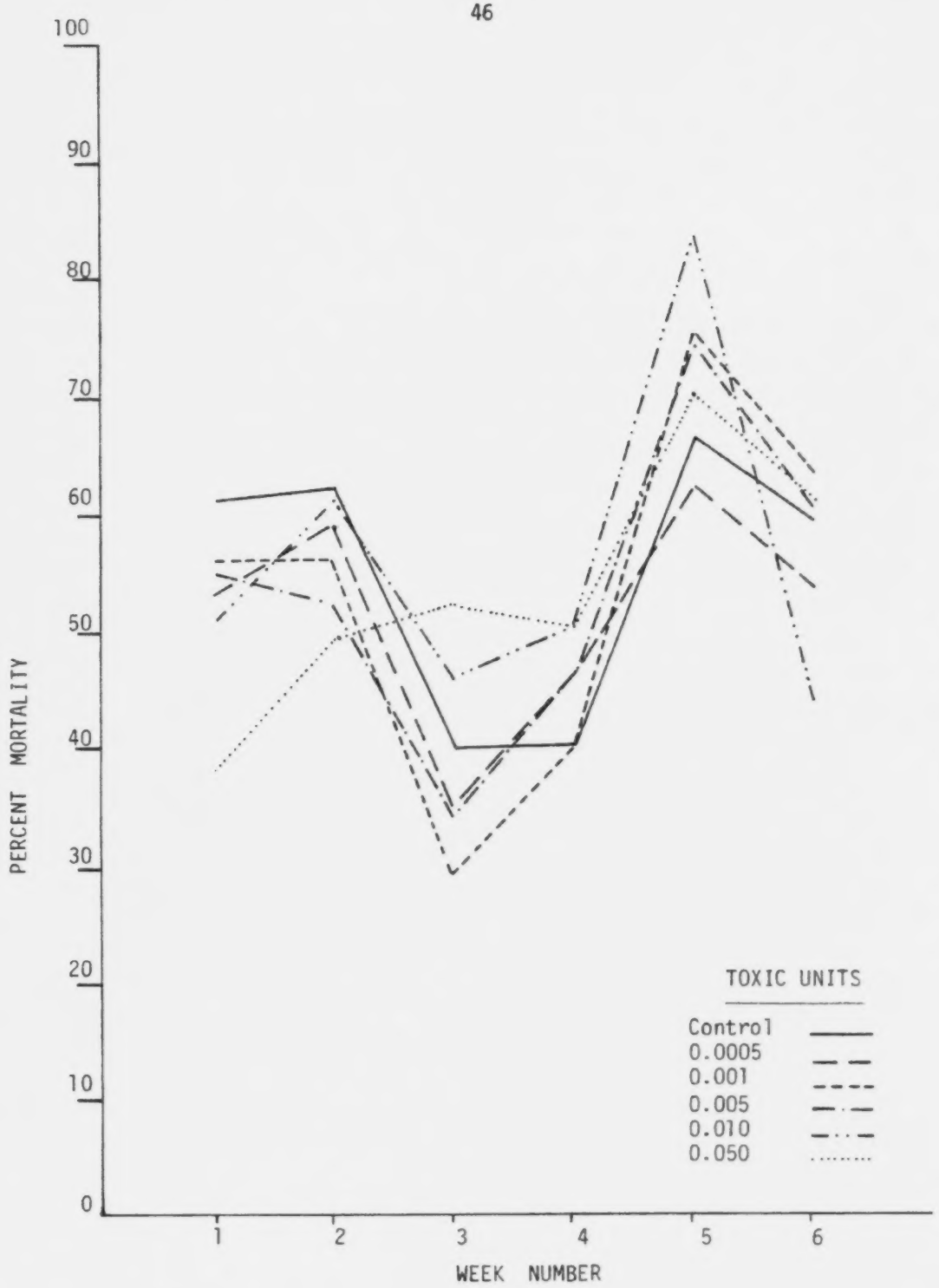


Figure 7

Average weekly mortalities as percent of survivors from each previous week. (replicates summed).

The percent weekly mortalities in the 1000 ℓ rearing tank (Table 2) show that initially, this tank exhibited very high survival, but its mortality rate rose to the same level as that of the sublethal treatments. Mortalities in the source stock for acute lethal and short-term sublethal tests were always below 10% per day. For the chronic sublethal tests, the level of background mortality was comparable in all effluent concentrations and controls.

Mortality rates observed here for larvae would be abnormally high for larger fish such as underyearling rainbow trout, but high mortality in larval herring is considered normal until they metamorphose into juveniles. Comparison with existing data on mortality of herring larvae in the field indicated that the overall mortality of larvae in the chronic sublethal tests was equivalent to field mortalities, regardless of whether causes of mortality were the same. The detailed study of herring larvae in Barkley Sound, B.C. by Stevenson (1962) led to the conclusion that between 98.9 and 99.7% of larvae in Barkley Sound die within six weeks. The average total percent mortality over six weeks in the chronic sublethal tests was 99.2%, within the range of mortality reported by Stevenson.

Mortality rates were equivalent between treatments in the chronic sublethal tests except in the highest test concentration (0.05 Toxic Units) which did not follow the pattern of other treatments during the first three weeks. Specifically, dead larvae were located visually and removed by pipette on days 1 - 10 (when larvae often rest near the bottom) whereas from day 11 on, the dead were siphoned out and sieved. In the acute tests it was noted that dead larvae decomposed, i.e. became opaque more slowly in BKME concentrations of 0.05 and 0.1 Toxic Units. Colour properties of higher BKME concentrations also made dead larvae more difficult to see, and it seems likely that in 0.05 Toxic Units BKME, a portion of mortalities occurring during weeks one and two were missed by visual pipetting and were subsequently taken up by siphon late in week two or during week three, resulting in the difference in mortality pattern.

CONCLUSIONS

In the acute lethal bioassays, larval stages of herring were more than twice as sensitive to BKME as threespine stickleback or underyearling rainbow trout. The chronic sublethal bioassay with larval herring revealed no toxic effects on growth or survival during a six week period, at concentrations within an order of magnitude of the 96 h LC50 for the larval herring. Thus, the thresholds for lethal versus sublethal effects may occur within a very narrow range of concentrations.

Considering the typical occurrence of a substantial baseline mortality rate among larval herring, replicates in acute lethal tests may be one way to determine whether meaningful results can be obtained. Testing larvae from different parental stocks could also provide useful insights, as would the use of a reference toxicant.

A glass jar of one liter volume appeared to disrupt normal feeding behaviour in larval herring. Planktonic organisms have not evolved any capacity for negotiating solid obstacles, so herring larvae tended not to turn around when they swam into the wall of a container. If they did turn around, their expression of normal feeding appeared to have been suppressed.

The difference observed between larvae in the stock tank (flow-through, 1000 ℓ) and in the chronic sublethal tanks (static 50 ℓ) also suggested disruption of normal behaviour. The basic differences were in the growth and survival rates during the yolk stage. In the stock tank, in which larvae seldom came close to tank walls, feeding was initiated in most fish on day 2, well before the end of the yolk stage. Thus, during the yolk stage the larvae in the stock tank derived energy from both internal and external sources and exhibited rapid growth as well as low mortalities. In the 50 ℓ sublethal exposure tanks, larvae were observed to contact tank walls frequently and their initiation of feeding occurred more slowly, possibly owing to behavioural disturbances from contacts. The process of transferring newly hatched larvae from the stock tank to 50 ℓ tanks may also have disturbed initiation of feeding. It can be concluded that the smaller the container, the more frequent the wall contacts and the lower the level of feeding. Therefore, there is a need to develop effective static rearing containers small enough for mass use in bioassay tests on larval herring.

Aside from test container designs, handling procedures such as use of pipettes (Wilson, 1972 and 1977) and microscopic examination for heartbeat (Blaxter, 1977; Wilson, 1977) might also be presumed to disturb behaviour. In our rearing studies, any manipulation involving direct contacts to larvae or lasting more than a few seconds resulted in fatality. Larvae must be transferred quickly in a small volume of water with minimal turbulence. Similarly, the determination of death was based on the change

from translucency to opacity in the present study, a criterion which involved no manipulations. Experiments exclusively using starved yolk-sac larvae might be viewed with the suspicion that the organisms were moribund. Procedures should be included to demonstrate viability.

The criterion for assessing acceptable holding and handling procedures in acute lethal bioassay should be that control organisms be able to resume feeding and display growth after the end of the test period. This would be a simple and reasonable criterion for acute lethal tests with rainbow trout fingerlings, for example; five days of starvation in a small aquarium should not seriously affect a juvenile fish. As mentioned, many species of larval marine fishes tend not to recover normal behaviour and development after almost any physical manipulation, even without direct contact. This consideration underlines the physical sensitivity of such larval forms but should not argue against their use as relevant test organisms. The importance of larval fishes to marine ecosystems and to fisheries management dictates that they be investigated by toxicologists, but evidence of their sensitivity to physical disturbances further dictates special attention to procedural operations in order that viability can be demonstrated.

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ABSTRACT

Ketcham*, D. 1978. Marine Environmental Impact Monitoring of Kraft Mill, Paper Machine and Woodmill Effluents. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

MacMillan Bloedel Limited has undertaken extensive marine monitoring programs of the impact of effluent discharges on the receiving waters from its three B.C. pulp and paper operations - Harmac, Powell River, Port Alberni, as well as Chemainus Sawmill operation.

This paper presents and discusses the studies conducted over the past six years and demonstrates the site specific characteristics of the impact of effluent discharge.

Survey methods employed during the studies are discussed and include chemical and physical water quality testing, plankton analyses, benthic and intertidal sampling. Through the use of marine monitoring programs, it has been possible to not only determine the impact and zones of influence of effluent discharge from the above operations, but also to indicate improvement in receiving water quality and ecology following implementation of pollution abatement measures. These monitoring studies provide an indication of what further improvements of effluent discharge quality may be required to minimize or eliminate impact on the environment.

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THE BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF SUSPENDED SEDIMENT ON JUVENILE SALMONIDS

by

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ABSTRACT

Noggle, C. 1978. The behavioral and physiological effects of suspended sediment on juvenile salmonids. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C. Nov. 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Studies were conducted to assess the effects of suspended sediment upon juvenile salmonids in the stream environment. Static bioassay tanks were used to determine 96 hour LC50's and changes in blood physiology. Two experiemntal stream designs were used to relate sediment concentrations to avoidance behavior.

Results indicate seasonal changes in the tolerance of salmonids to suspended sediment. Bioassays conducted in autumn showed LC50's in excess of 30,000 mg/l; summer LC50's were less than 2,000 mg/l. Blood glucose levels were elevated at sublethal suspended sediment concentrations. Experiments conducted with a turbid artificial stream and clear tributary indicate a reluctance by the fish to leave their established territories. Studies conducted with a Y-shaped stream show a preference for turbid water at medium concentrations and slight avoidance at high concentrations.

INTRODUCTION

Many of man's land use activities cause suspended sediment concentrations in streams to increase (Cordone and Kelly 1960). Some of the major sediment sources include agriculture (Wallen 1951), mining, dredging (Sherk et al. 1974), road construction and logging. These activities have all provided the need for studies of the effects of suspended sediment on fish.

My particular study is part of a long-term project to assess the effects of logging on the Olympic Peninsula of Washington. Experiments were conducted at Clearwater Camp, 43 kilometers south of the town of Forks.

MATERIALS AND METHODS

FISH AND WATER SUPPLY

Wild coho salmon (Oncorhynchus kisutch) and chinook salmon (Oncorhynchus tshawytscha) were collected from nearby streams with hand-held seines. Hatchery coho and steelhead trout (Salmo gairdneri) were obtained from the Washington State Sol Duc Hatchery and Bogachiel Rearing Pond, respectively. All fish were held unfed for one to five days within an in-stream live box at the study site prior to testing.

The water supply was taken from a nearby stream and had the following characteristics when sampled October 11, 1977: pH 6.9, conductance 48 μmhos/cm, alkalinity 9.3 ppm, and hardness 14.9 ppm.

AVOIDANCE STREAMS

One apparatus consisted of two 1.2 x 9 meter artificial streams into which sediment was added, and each had a simulated clear-water tributary. The suspended sediment concentration was maintained in the artificial streams by recirculating mainstream water with electric pumps and continuous slurry addition to compensate for settling and dilution resulting from the clear tributary. The excess water added by the tributary was removed by an overflow drain on the sump. Suspended sediment concentrations were monitored by hourly sampling coupled with continuous flow turbidimeter recordings.

Thirty fish were held between screens located one meter above the tributary entrance and at the downstream end of the spill. This area contained three pools 1.8 meters long and 30 centimeters deep, separated by riffles, six centimeters deep. Avoidance behavior was quantified by comparing the number of fish in the tributary of the turbid stream to the number of fish in the tributary of the clear (control) stream at half-hour intervals for the two-to-ten-hour duration of each test. Downstream avoidance was determined by removing the spill screens after sediment addition began and comparing the number of fish to drop into the net-lined sumps. Feeding efficiency was determined by tossing 45 shelled caddisfly larvae, one at a time, into the downstream pool containing 15 wild coho smolts. The fish were immediately sacrificed and the total number of caddisfly larvae eaten was recorded. Twelve avoidance tests (ten with coho, two with steelhead) and five feeding tests (four in turbid waters, one in clear water) were conducted.

Y-TROUGH

The second apparatus was a Y-shaped trough 2.5 meters long, 23 centimeters wide, and 13 centimeters deep, used to allow coho a choice between turbid and clear water. The flow was adjusted to approximately 0.6 liters per second in each arm. Sediment was added to one arm and recorded with the continuous flow turbidimeter, calibrated with a weighed grab sample.

About one-third of the Y-trials were conducted with a plywood sheet over the two arms and upper half of the main stem to determine if cover affected choice. Removable screens held a group of five coho for the five minute acclimation period at the outlet end of the trough. The upstream screen was then removed and the fish were allowed to move freely within the apparatus. At the end of one-half hour, screens were placed at the intersection of the arms. The number of fish in the turbid arm, clear arm, and mixed main stem were recorded for each of the 42 trials conducted.

BIOASSAY TANKS

The third apparatus, used to conduct static bioassays, consisted of six cylindrical 60-liter containers with a 125° cone sealed into the bottom. The test suspension was continuously withdrawn from the bottom of the cone, through a 16 liter/min. pump, and spilled onto the surface of the suspension. This effectively oxygenated and stirred the sediment. The tanks were placed in a nearby stream in 20 centimeters of water to maintain the natural temperature regime.

Tanks were sampled for mortality and suspended sediment one to four times daily, depending on the rates of sediment settling and mortality. At the end of 96 hours the final samples were taken and the survivors were sacrificed to determine blood hematocrit, and plasma chloride and glucose concentrations.

Y-trial results were analyzed with chi-square calculations. Blood physiology results were analyzed using a one-tailed student's t-test. A detailed description of methods will be available in Noggle (in press).

RESULTS

STREAM AVOIDANCE

The stream avoidance study showed no significant avoidance either downstream (dropout) or into the clear tributary, even though fish were exposed to suspended sediment concentrations (2,500 mg/ℓ in spring) approaching the 96 hour LC50 levels.

The results of the feeding experiment indicate a steady decline in the ability of coho salmon smolts to feed as the suspended sediment concentration increases (Fig. 1). From a maximum feeding rate in clear water, feeding decreases to zero at about 300 milligrams per liter.

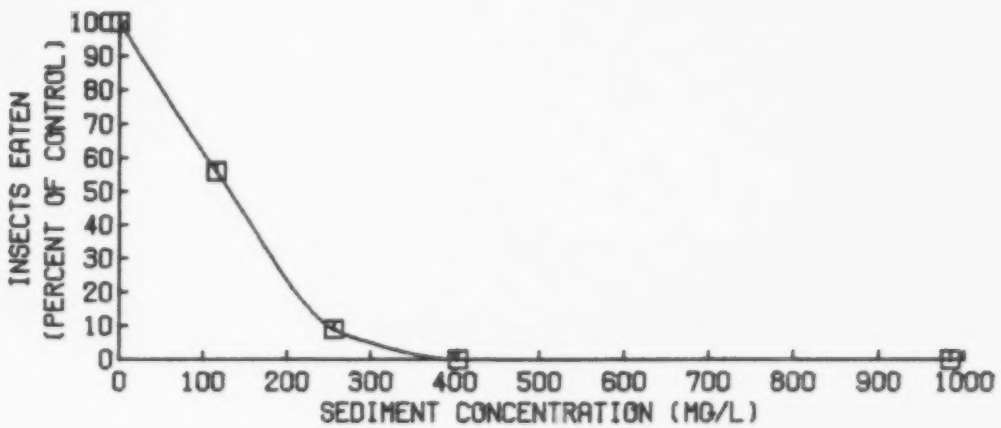

Fig. 1. Number of caddisfly larvae eaten by 15 coho versus suspended sediment concentration.

Y-TROUGH

The Y-trial resulted in a significant ($p<0.001$) shift toward the downstream arm of mixed turbid and clear water at low concentrations (Table 1). At high concentrations, at least two to four times the 96 hour LC50, coho begin to show a significantly ($p<0.005$) stronger selection for the clear arm, yet the majority still preferred the mixed zone. There were no significant differences between covered and uncovered Y trials.

Table 1. Percentage of wild coho choosing the turbid, mixed, and clear Y apparatus arms, between July 18 and September 10, 1977.

Sediment Concentration (mg/ℓ)	Percentage per arm			No. of coho
	Turbid	Mixed	Clear	
0	34%	30%	36%	50
1,000-4,000	23%	71%	6%	65
4,000-12,000	15%	65%	20%	95
				210

BIOASSAY AND PHYSIOLOGY

Static bioassays indicated a wide range of tolerance levels. Coho LC50's varied by a factor of greater than 20 between late summer (August 20) and mid-autumn (November 10) (Fig. 2, Table 2).

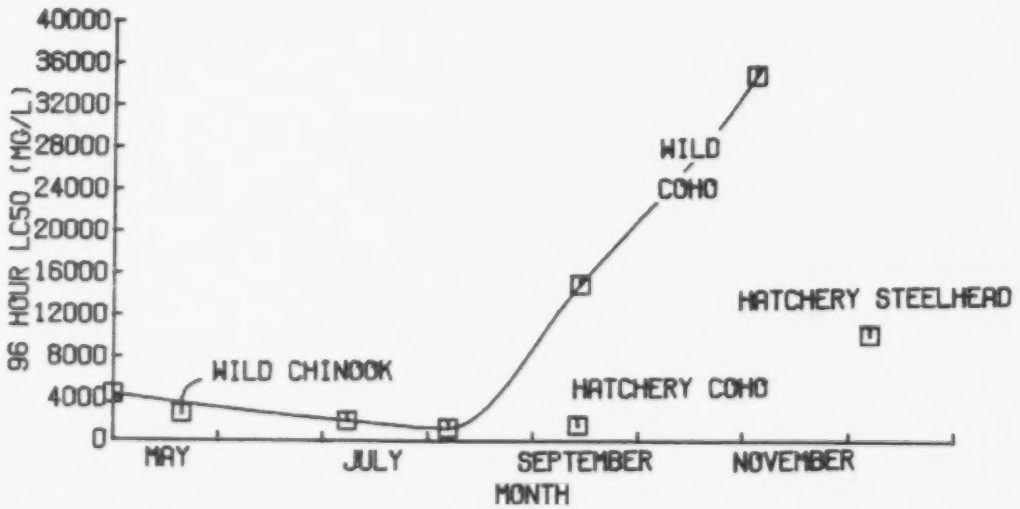

Fig. 2. Seasonal change in wild coho 96 hour LC50's compared to LC50's for wild chinook, hatchery coho, and hatchery steelhead.

Table 2. Test conditions and results of LC50 bioassays.

Date	Species	Origin	Length (mm)	Fish / Tank	Grams / Liter	Temp (°C)	No. of Tanks	96 hr LC50	0.95 Conf. Int.
5/1	coho	wild	132	10	4.13	7	6	4,420	2,739-7,327
5/20	chinook	wild	51	50	0.85	11	10	2,586	2,391-2,803
7/7	coho	wild	62	10	0.85	14	17	1,927	912-4,422
8/6	coho	wild	75	10	0.92	15	5	1,198	591-2,622
9/13	coho	hatchery	62	10	0.53	12	3	1,500	*
9/13	coho	wild	72	10	0.85	12	3	15,000	*
11/13	coho	wild	83	10	1.05	8	11	35,000	*
12/6	steelhead	hatchery	140	5	2.50	6	10	10,233	6,774-16,222

*LC50 estimates too approximate to estimate 0.95 confidence intervals.

The blood physiology results suggested stress occurred at sublethal suspended sediment concentrations. Hematocrit levels show no relationship with sediment concentrations (Table 3). Plasma chloride concentrations showed no consistent trend in initial autumn samples and were discontinued in spring samples due to small blood volumes in age 0 fish. Plasma glucose concentrations were higher in coho exposed to suspended sediment. Low (<.2 x LC50), medium (>.2 x LC50 and <.8 x LC50), and high (>.8 x LC50) sediment exposures all produced significantly different plasma glucose concentrations.

Table 3. Blood hematocrit and plasma glucose concentrations for coho held 96 hours at low, medium and high suspended sediment concentrations.

Sediment Concentration (Proportion of LC50)	Hematocrit(Vol%)	Glucose(mg%)	No. of Coho
Low (<.2)	41.4	75.4	120
Medium (.2<x<.8)	43.2	82.4	65
High (>.8)	43.0	99.9	29
			214

DISCUSSION

The results from the stream avoidance and Y-trial experiments suggest that juvenile stream-rearing salmonids do not avoid water with suspended sediment concentrations several times greater than what generally occurs in nature. Some of the more important natural selection pressures responsible for this behavior may include the advantage of maintaining the home territory (Chapman 1966), the risk of physical injury when migrating through extremely turbid water during high flows, the cost of establishing a territory over prior residents if a suitable stream is found (Hartman 1965), and the costs and probability of failing to relocate its previous territory. The feeding study indicates that feeding is reduced at relatively low turbidities. This could be one of the more serious effects of land use activities in which chronic low level turbidity levels result.

Suspended sediment concentrations occurring naturally probably rarely approach lethal levels, although lethal levels found here (1,500 35,000 mg/ℓ) are much lower than the values (>100,000 mg/ℓ) reported in most previous studies (see reviews by Cordone and Kelly, 1960; Sorenson, et al., 1977). Natural suspended sediment concentrations show the same seasonal trend as the 96 hour LC50's. The observed LC50's are several times higher than the turbidities observed in Olympic Peninsula streams at the same time of the year. Man-induced turbidity, out of phase with the natural salmonid tolerance cycle, could adversely affect juvenile salmonids. For example, extremely turbid waters in late summer could stress salmonids.

Observed 96 hour LC50 differences between coho, chinook, and steelhead seem to be minor. Seasonal and hatchery versus wild differences appear to be much greater. Blood physiology results suggest plasma glucose measurements show promise as an indicator of sublethal stress induced by suspended sediment.

Future studies should define the threshold effect (EC50), measured by hyperglycemic response as in McLeay's (1977) study of stressful levels of pulpmill effluent. Additional stress indicators such as blood leucocrit (McLeay and Gordon 1977) and plasma hormone (Mazeaud et al. 1977) levels may provide more insight into the sublethal effects of suspended sediment.

Rogers (1969) and others have suggested that the primary mechanisms of turbidity stress are physical abrasion of gill lamellae and gill mucous "clogging," causing anoxia. Suspended sediment studies incorporating a closer look at gill abrasion and clogging using histological methods, with bioassays designed to measure the effects of water temperature and prior sediment exposure should improve understanding the observed seasonal tolerance changes.

Comparative studies of the effects of various sediment sources to understand the importance of sediment particle angularity and size distribution are also important. All of this information is necessary to develop a sufficiently detailed model to predict the stressful or lethal effects of a particular suspended sediment concentration upon a particular population of salmonids.

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ABSTRACT

Salo, E.O. and B.W. Snyder*. 1978. Field and Laboratory Program on Hood Canal, Washington. Proc. Fourth Annual Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

For the past three years, the Fisheries Research Institute of the University of Washington has been monitoring the effects of pier and drydock construction, including dredging, associated with the U.S. Navy TRIDENT submarine base at Bangor on Hood Canal, Washington. The research included the use of a self-contained floating laboratory with static and flow-through systems. The emphasis has been on juvenile salmonids, especially the chum salmon, Oncorhynchus keta. Bioassays included avoidance behaviour. Integrated field work included sampling by tow-net, beach seine, submerged live boxes and a mark and recovery program. The effects of lighting were studied by acoustics with the transducers placed on the bottom near the lighted piers. The condition of the fish, both hatchery and feral, had a significant influence upon their tolerance to sediments and upon their behaviour. Vibriosis was the most important disease and this year's work included testing of immunized fish.

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EXPERIMENTAL ECOSYSTEMS AS A MEANS OF EVALUATING THE FATE AND EFFECT OF CONTAMINANTS IN AQUATIC ECOSYSTEMS

by

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ABSTRACT

Hodson, Peter V. and E. Scott Millard. 1978. Experimental ecosystems as a means of evaluating the fate and effect of contaminants in aquatic ecosystems. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Studies of contaminants using a single species do not illustrate the potential for biomagnification, persistence or toxicity in a complex ecosystem. Studies of existing contaminated ecosystems are expensive, time consuming and difficult to accomplish due to the high degree of complexity and size of natural ecosystems. To bridge this gap, increasing use is being made of small, experimental ecosystems to evaluate contaminant fate and impact. These range from small aquaria in the lab to large enclosures of water in the field. These approaches are reviewed and the utility of results discussed, with some illustrations of experiences at the Great Lakes Biolimnology Laboratory. When the limitations of these methods and their proper role in decision-making is recognized, they provide a fast, valuable means for preliminary assessment of environmental hazard.

Environmental problems due to toxic substances are increasing in number at a faster pace than are studies and assessments of individual toxicants. Each year new contaminants are measured in fish at levels rendering them unsuitable for consumption and fish populations decline under the influence of fishing pressure, habitat disruption and the subtle detrimental effects of sublethal exposure to a variety of toxic substances.

Assessment of environmental hazard of a chemical should include studies of lethal and sublethal toxicity to aquatic organisms plus potential for biomagnification. Current methodology includes lab studies of organism life cycles and extensive surveys of toxicant concentrations in natural ecosystems. Both approaches are very costly in terms of time, manpower and money. Consequently, response to new crises is slow. The passage of the Environmental Contaminants Act has increased the demands for new information by requiring a screening of all new chemicals to prevent future crises. A response to this pressure is the proposal for 60-day bioassays of larval fish to assess toxicity, since this life stage is generally the most sensitive (McKim, 1977).

Another approach is that of model ecosystems. They are assemblages of two or more trophic levels that interact, usually through predation, and which are exposed to a substance to measure:

(a) accumulation and associated rate constants for uptake and transfer from water and/or diet

(b) concentration factors between compartments

(c) rate of degradation and character of degradation products

and (d) toxicity.

There are two approaches to model ecosystems, synthetic and natural. Synthetic systems have few species or trophic levels, are "built" species by species, have a very definite, short lifetime, and often have unrealistic ratios of predators to prey. These systems do not "model" or "imitate" specific ecosystems but include some of their characteristics, such as predator-prey relationships, transfer of energy, biomass and contaminants, primary producers, consumers and degraders. Biomagnification and degradability of compounds like DDT have been shown to be qualitatively similar to that observed in the field (Metcalf et al., 1971).

The advantages of this approach (Table 1) are speed, repeatability and simplicity. However, due to a lack of complexity, balance and longevity, degree of hazard may be over or underestimated, and rate constants are not reliable enough for modelling real ecosystems.

TABLE 1

SYNTHETIC MODEL ECOSYSTEMS

ADVANTAGES	DISADVANTAGES
1. Speed and cost.	1. The experimental ecosystems are often oversimplified and unbalanced resulting in:
2. Results reflect observed behaviour of chemicals in field studies.	a. unrealistic rate constants for contaminants transfer
3. Simple ecological interactions allow better understanding of contaminant transfer.	b. "forcing" of contaminants through specific predator-prey links to give unrealistic distribution
4. More easily replicated and repeated within and between labs than field studies.	c. misleading ecological magnification indices.
5. Information produced on toxicology, biomagnification, biodegradation, transport and functional group analysis of contaminants.	2. Equilibria and effects requiring long-term experiments cannot be measured.
6. Sites of biodegradation can be identified.	3. Requirement for radioisotopes prevents detection of unlabelled breakdown products.
7. Mass balance of contaminants can be computed.	

Toxicity may not be adequately assessed since the test biota are those adapted to lab conditions. Since the behaviour of a contaminant in a real ecosystem will vary with its characteristics, time of year, source of chemical, etc., the synthetic approach only provides a relative idea of behaviour and a rough ranking of degree of environmental hazard.

An additional limitation in small systems is the requirement for the use of radio labelled contaminants. Labelling contaminants permits greater analytical sensitivity, smaller sample sizes and faster analyses. These benefits may be offset by an inability to detect hazardous, non-labelled breakdown products.

Natural model ecosystems are those "modelling" or "imitating" an existing ecosystem. Generally, a part of this existing ecosystem is studied in situ under natural light levels, photoperiods, temperatures, etc., or removed entirely to the lab where these variables are controlled.

A review of the advantages and disadvantages of natural ecosystems (Table 2) indicates that while they provide fairly realistic models of known ecosystems and contaminants dynamics, they are generally large, expensive, time consuming, are less repeatable and more difficult to understand than synthetic model systems. However, they are intermediate in all respects between synthetic ecosystems and a full field study.

The following are descriptions of typical model ecosystems, progressing from simple to more complex types.

I. Small Volume Aquaria

(a) The simplest model ecosystem is synthetic and contains one species each of algae, zooplankton and fish. These can be exposed to contaminants either individually or combined in a food chain according to Figure 1. A modified version has been described by Terhaar et al. (1977). At each trophic level, this system can identify bioaccumulation from water and food by direct measurement and subtraction (Figure 1). The system could be made larger and more complex. However, the difficulties in sustaining large scale, constant primary and secondary productivity to feed a realistic density of fish renders large or long-term experiments impractical. In addition, low flows, dissolved nutrients, and high plankton densities dramatically reduce contaminant concentrations in water (Terhaar et al. 1977). Consequently, measurement of toxicity and contaminant uptake from water are confounded.

(b) Metcalf model ecosystem

One of the best known and most widely used model ecosystems is the synthetic system of Metcalf and associates at the University of Illinois (Metcalf et al 1971; 1973). Their system includes both a terrestrial phase as a source of pesticide and an aquatic phase as the area to be analyzed. The pesticide is released to the aquatic

TABLE 2

MODEL ECOSYSTEMS WITH NATURAL ASSEMBLAGES

ADVANTAGES	DISADVANTAGES
1. More realistic - may draw conclusions about specific ecosystems with specific problems.	1. Expensive, time consuming and require large facilities.
2. Rate constants, ecological magnification factors and biodegradability indices should be closer to observed field data.	2. Complexity creates difficulty in understanding routes and mechanisms of contaminant transfer and sites of biodegradation.
3. Greater chance of identifying sensitive species since more are tested.	3. Difficult to replicate between and within labs. In a series of identical units, each may deviate in a different way from the same starting point.
4. Greater variety of organisms and hence numerous possible pathways of contaminant transfer.	4. Separating organism response from natural "drift" and variability is difficult.

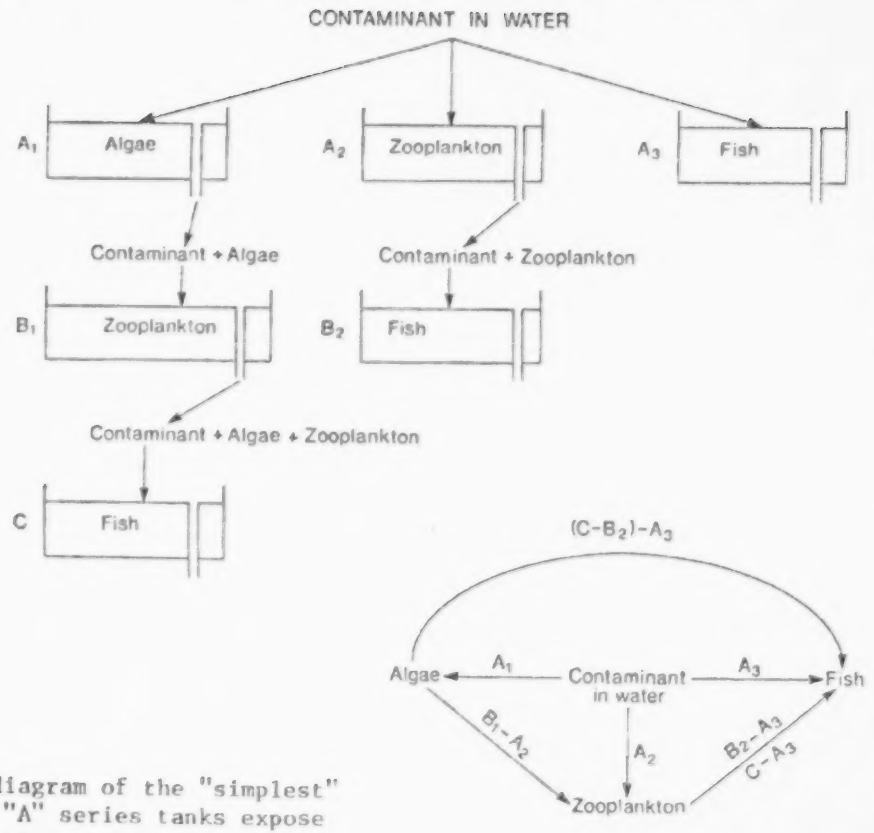


Figure 1. Schematic diagram of the "simplest" model ecosystem. The "A" series tanks expose single species to waterborne contaminants only. Overflow from the algal and zooplankton tanks provides waterborne and dietary contaminant to the "B" and "C" series tanks. Contaminant uptake from water is measured directly in the "A" series while uptake from food is calculated by substracting observed concentrations in "A" from observed concentrations in "B" and "C".

phase by leaching and bacterial action on leaf litter and herbivore faecal pellets. This system is assembled and analyzed sequentially (figure 2 & Table 3). Predator levels are excessive so that all prey is consumed and the experiments are short.

TABLE 3

FLOW CHART - METCALF MODEL ECOSYSTEM

Days		
0		Construct aquatic-terrestrial ecosystem with - washed white sand - 7 liters standard reference water - 50 sorghum seeds (Sorghum vulgare) - 30 Daphnia magna - 10 snails (Physa sp.) - mixed plankton (algae and protozoa)
20		Add pesticide to plants (~ 15 cm high) at a rate of 1-5 mg in acetone, 5 µl per plant (= 0.2-1 lb/acre)
21	Measure pesticide in water	Add ten 4th instar saltmarsh caterpillars (Estigmene acrea)
26		Remove a sample of Daphnia magna for analysis
		Add 300 mosquito larvae (Culex pipiens quinquefasciatus)
30		Remove 50 mosquito larvae for analysis
		Add 3 mosquito fish (Gambusia affinis)
33		Terminate experiment - measure radioactivity of fish, snails, mosquito larvae, algae and water.

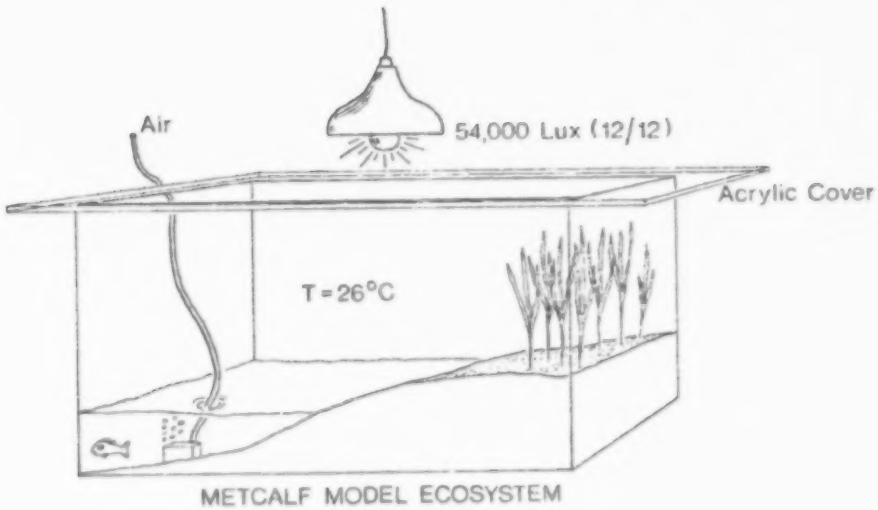
Calculate ecological magnification (EM) = $\frac{\text{conc'n of compound in organisms}}{\text{concentration in water}}$

Biodegradability index (BI) in organism

$$= \frac{\text{conc'n of radiolabelled polar degradation products}}{\text{conc'n of radiolabelled non-polar degradation products}}$$

Unextractable radioactivity = indication of total degradation
- low for poorly degraded materials and high for materials degrading quickly to gas.

In this system, persistent (DDT) and labile (organophosphates) compounds have exhibited the same characteristics of bioaccumulation and biodegradation as observed in field studies. Consequently, DDT is used as a reference to assess the relative environmental hazards of test compounds. The Metcalf system is a useful screening tool but



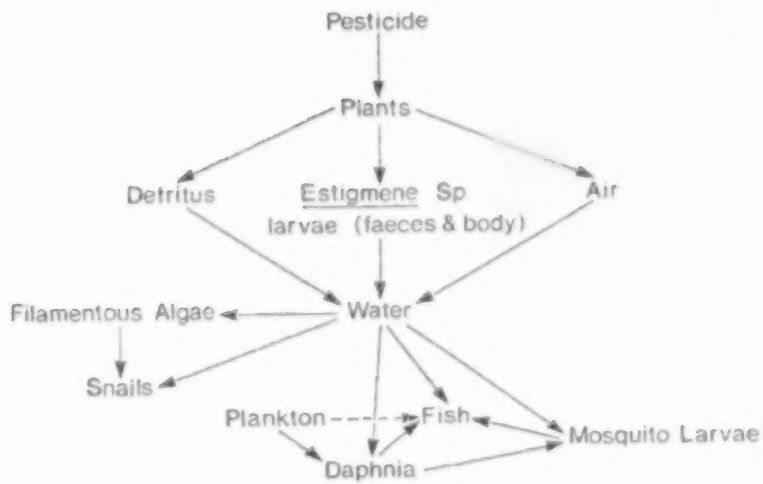


Figure 2. The Metcalf model ecosystem. The food chain illustrates possible pathways by which contaminants may be transferred from the plants on the terrestrial phase to the organisms of the aquatic phase.

suffers all of the faults of the synthetic approach due to its highly artificial nature.

II. LARGE VOLUME LAKE SIMULATORS

(a) Lake Column Simulators

Lake Column simulators are cylindrical, high-volume, indoor enclosures that attempt to simulate the open-water conditions in a lake (Figure 3).

The columns in use at the Great Lakes Biolimnology Laboratory are 4 m high, 1 m in diameter and have a volume of ca 3500 liters. Radiant energy to each column is supplied by a 1,000 W tungsten-halogen lamp on a 12 H photoperiod. The water in the upper half or epilimnion of each column takes on the ambient air temperature while the lower half is cooled to 12 c to create stratification and a hypolimnion. Artificial mixing of the epilimnion is provided by pumping surface water down through a 1 m u-shaped tube that is directed back towards the surface. Productivity is manipulated by nutrient additions allowing study of both contaminants and nutrient problems or their interaction.

Both synthetic and natural approaches can be used to establish planktonic plant and animal communities. Lake water containing natural assemblages of organisms is used to fill the columns in the natural approach. Although environmental factors can be controlled in the columns, organisms from the lake are subjected to a very different set of growth conditions than in the lake. As a result, a succession occurs to less diverse communities, better suited to the imposed conditions, resulting in communities unrepresentative of the lake. Species that later become dominant were often originally rare or sub-dominant. The inoculum varies seasonally and changes to lab conditions are most severe in the winter. Experiments with natural communities should be short if results are to be related to the lake environment.

In the synthetic approach, the columns are filled with tap water and nutrients and innoculated with algae and zooplankton from large indoor, batch cultures of mixed species. The inoculum undergoes only limited changes in conditions from culture to column. Communities are established faster and more easily and events are more predictable than with the natural approach. However, both phytoplankton and zooplankton communities lack diversity and are not always representative of lakes. Biota, and hence results, must generally be regarded as exhibiting the functional aspects only of planktonic communities. These systems have been used to study effects of atrazine, PCB's and nutrient loading on column communities.

(b) Open-water Enclosures

Many studies have used various shapes and sizes of enclosures

to isolate fresh or salt water (Antia et al. 1963; Lack and Lund, 1974; Lean et al. 1975 and Takahashi et al. 1975). Limnocorrals, for example, consist of plasticized curtains zipped together in a triangular shape. They are suspended from the surface of lakes by styrofoam floats and the lower edge may be embedded in the sediment (Lean et al. 1975) (Figure 4) or left hanging free in the water column.

Limnocorrals offer natural light and temperature regimes but variable climatic conditions can make replication and prediction poor. Inclusion of the sediment-water interphase offers realism, but processes at this boundary are often dependent on turbulence and vertical mixing which are modified by the enclosure walls (Boyce, 1974). The phytoplankton communities of separate limnocorrals tend to diverge with time from one another and from those in the surrounding water. As with the lake column simulators, treatments should be started soon after installation and hypotheses formulated with regard to effects of treatments on the initially enclosed communities. The longer enclosures are established, the more general are the types of questions that can be asked and the less relevant the results are to the surrounding body of water.

(c) Littoral Zone Simulators

These systems used in our lab model the highly productive littoral zone of lakes and are intermediate between model streams and model lakes. A continuous flow of water at 1 ℓ/min to tanks 40 x 150 x 60 cm high provides a 95% turnover time of 14 hours (Figure 5). One-half of the tank is illuminated as a zone of productivity while the shaded other half is a zone of degradation. Natural substrates and lake organisms are the components. Parameters measured are overall diversity plus numbers, species, biomass and contamination of benthos, protozoans, coelenterates, snails and algae. Sticklebacks (Gasterosteus aculeatus) consume oligochaetes, protozoans, zooplankton and algae and breed in filamentous algae. The system is maintained through primary productivity supplemented by "allochthonous input" of 40 g powdered leaf matter per month (equivalent to 800 $g/m^2/y$). We have studied contaminants dynamics of TFM* and the impact of a mixture of metals on these systems. Initial data indicate that there are no sudden peaks and crashes of any species. However, fish apply selective pressure to specific food organisms so that non-fish food items increase in relative abundance and fish food items change in species composition as the fish grow. The principal difficulties encountered have been devising methods to easily measure numbers, species, contamination and performance of organisms.

III STREAM SIMULATORS

(a) Indoor

A stream channel 13 feet long and 4 feet wide has been

*TFM - 3-trifluoromethyl-4-nitrophenol, a lampricide.

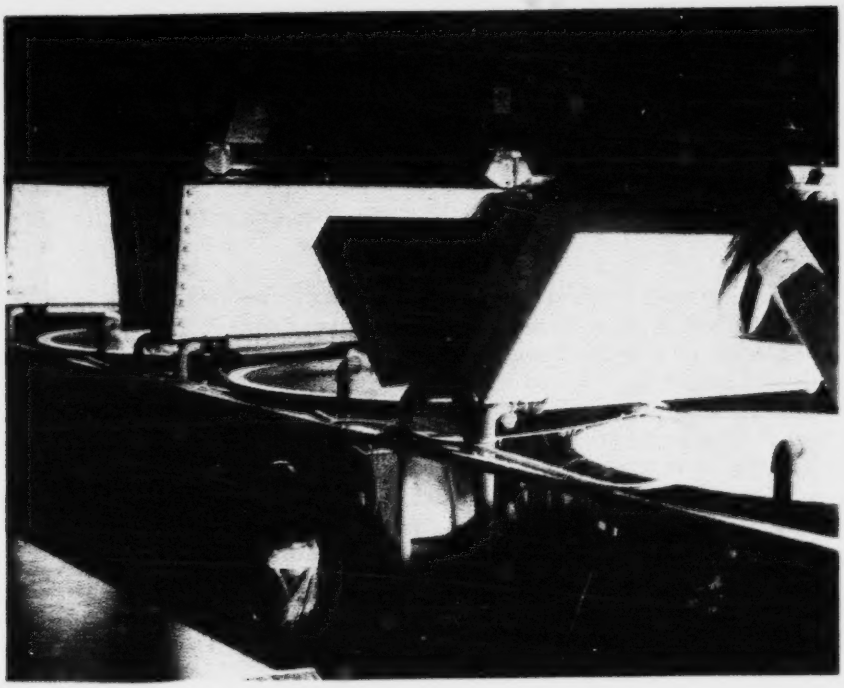


Figure 3. Lake column simulators.

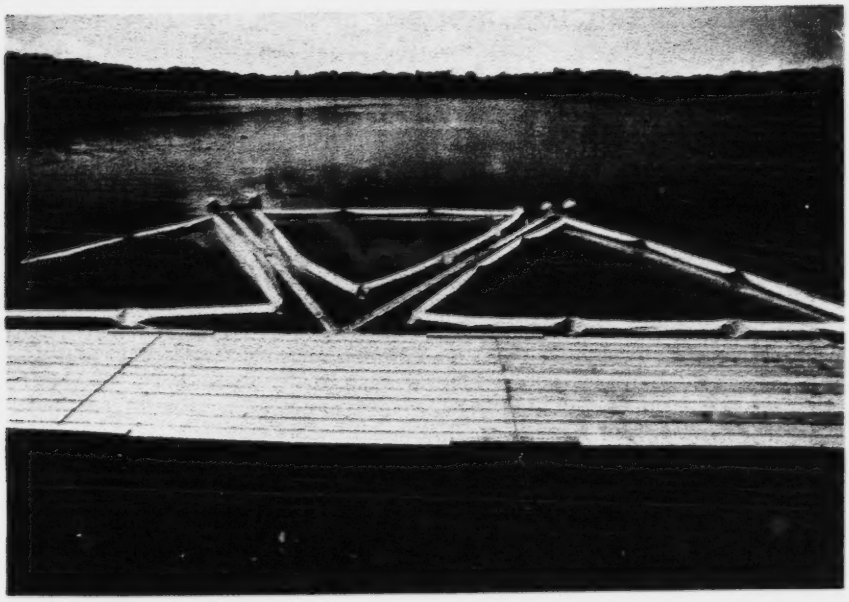
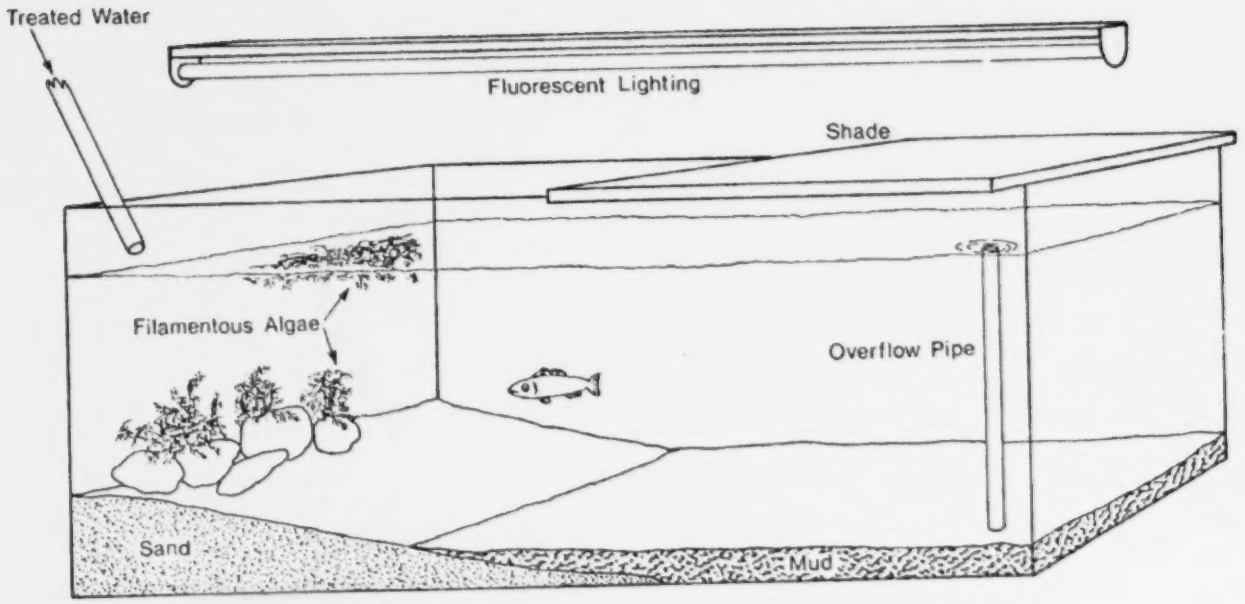


Figure 4. Top edge of 3 triangular limnocorrals suspended in the Bay of Quinte.



BIOTA: Filamentous algae, bacteria, fungi
Periphyton (diatoms, protozoans, hydra)
Snails
Oligochaetes, Gammarus sp., Asellus sp.
Fish

Figure 5. Diagram of a littoral zone simulator.

established in our lab to provide post-exposure stress for intoxicated fish (Figure 6). A divider down the centre creates a longitudinal "donut" and gives two channels per tank with water circulation by centrifugal pump. High water current, limited food and competition are used to identify "weak" fish through changes in behaviour and stored energy. Substrate is from a natural stream. The system could be adapted to other studies of biomagnification of contaminants but the cost and size reduces the opportunity for replication. Brusven (1973) has described a similar laboratory stream for studying insect-fish-substrate relationships that would be adaptable to studying contaminants. His system is 5 ft. long, 40 inches wide and 9 inches high. The current controls species distribution, especially of fish. Conditions may be manipulated by adjustment of baffles and substrates to favour or discourage specific species and to make all parts of the channel uniform. Since such systems are small and relatively inexpensive they could be built in multiples for replication.

(b) Outdoor

Stream channels usually consist of natural stream substrates and aquatic communities in a man-made channel. Temperature, light level and photoperiod vary naturally and only flow is controlled.

Maki and Johnson (1976, 1977) studied impact of a 1-day exposure to TFM on respiration of benthos and TFM kinetics in stream channels established with natural substrate and biota in hatchery raceways at a light level of 9500 lux. Flow, primary productivity and community respiration in the model streams were very close to those of a natural analog - a small shaded woodland stream. TFM increased community respiration and decreased productivity of riffles. This trend towards heterotrophy was reversed within 2-3 days post-exposure. Stream species with relatively rigid exoskeletons or calcareous shells accumulated radiolabelled TFM to a lesser extent than those with soft membraneous exteriors. After TFM exposure, depuration was log-linear except in worms that continued to take up TFM from contaminated organic sediments. This may explain why tubificids are among the more sensitive invertebrates to TFM (Schnick, 1972).

Judging by ecosystem performance measurements, this system accurately modelled a real stream and the impact of TFM on stream communities. Subtle important interactions of the contaminant with stream biota occurred that would be undetectable in a stream. A disadvantage of this system would be the requirement for fairly large channels.

(c) Stream Enclosure

Stream enclosures partition an existing stream into several identifiable channels, some treated, some as controls. Eisele (1975) has described a continuous dosing system for natural streams. Upstream and downstream screenings limit large fish and invertebrate movements but there is a continuous washout and immigration of smaller biota. The

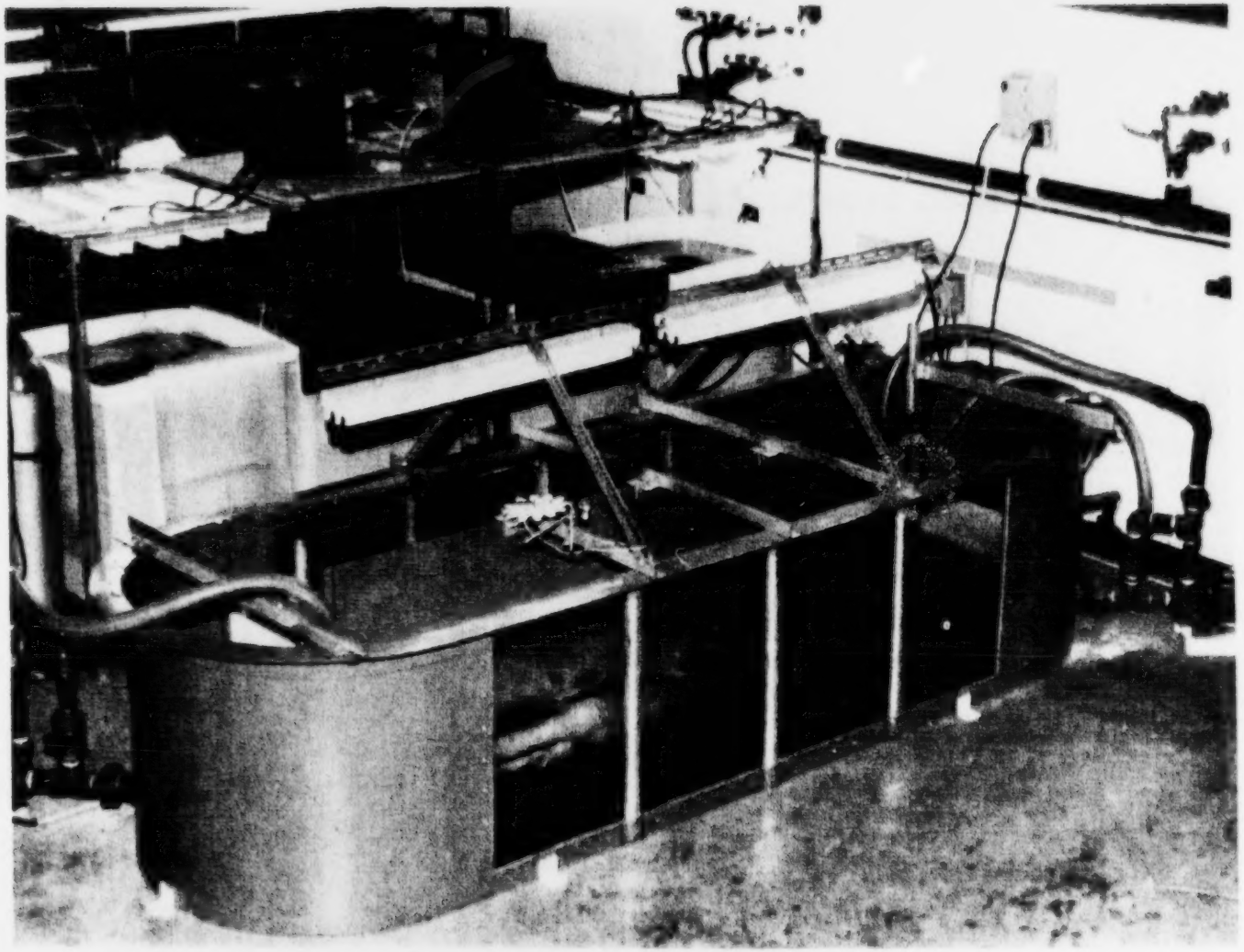


Figure 6. Photograph of a lab stream channel

result is a natural assemblage of organisms with a fairly close similarity between channels, natural variations or cycles of productivity, and a complex food web for contaminants transfer. The principle advantages of such a system are *in situ* measurements of real contaminants dynamics. In addition, upstream studies of a channel allow each to be its control. However, these systems require a high degree of ecological understanding and extensive background research. There is a chronic threat of flooding or drought that may ruin an experiment and contaminants are released into natural systems.

(d) Periphyton Communities

Studies of periphyton communities are popular because they limit complexity and focus on simple, nonmigratory organisms. They generally consist of a channel with a sand substrate receiving an input of unfiltered clean water. The established periphyton communities are characteristic of the supply water and may vary seasonally. The flow-through nature of the systems and the continued immigration of new organisms provides fairly constant community structure and good repeatability. Communities colonizing glass plates in each channel provide material for study.

Lab systems to study coal leachate have been discussed by Gerhart *et al.*(1977) while outdoor channels for zinc and mercury studies have been described by Williams and Mount (1965) and Sigmon *et al.* (1977). A potential disadvantage is that colonization of glass plate may not reflect long-term growth on the sides of channels (Williams and Mount, 1965).

FACTORS AFFECTING RESULTS

(a) Size and Shape of Containers

Size and shape of an enclosure are primary factors to consider. Large volume is required to produce large quantities of algal biomass to support more realistic population sizes at higher trophic levels. As an additional benefit, more biomass is made available for analysis of a contaminant residue.

Where open-water events are emphasized as in lake column simulators and limnocorrals, effect of vessel sides should and can be minimized, by initially considering the volume required, space available and the proper relationship of diameter to height that will yield low side-area to volume ratios. A cylinder is most often used for these studies because it has less side and bottom effect than an aquarium but offers an air-water interphase not available with a sphere.

For a cylinder of constant diameter, increasing the height increases both volume and side area at a constant and linear rate, the only net benefit being one of increased volume. In contrast, for a cylinder of constant height, increasing the diameter increases the side area linearly but the volume exponentially. The result is a beneficial

increase in volume and reduction of the side area:volume ratio. However, for any height of cylinder, increasing diameter beyond a certain point has comparatively little effect on the side:area to volume ratio but may produce a container with objectionable space requirements. For example; a column 4 m high and 1 m in diameter has a side area:volume ratio of ca 4 and increasing the diameter to 4 reduces the ratio markedly to ca 1 yet the diameter has to be increased to an unreasonable 16 m to reduce the ratio by another quarter to 0.25. An overiding factor will be that of cost. Increased container size usually decreases the total number of containers and hence the degree of replication and the number of treatments.

Both limnocorrals and lake column simulators are also plagued with the problem of undesirable displaced productivity from the open water to the sides in the form of attached algae. A few of the solutions to the problems that exist are:

1) introduction of a herbivore such as snails to control growth
2) allow periphyton to develop and include it in the study
3) routinely scrape material off the sides
4) establish dense phytoplankton populations early to shade out periphyton
5) keep experiments short (6 weeks maximum).

The first two solutions are least desirable because they create additional work, complicate the situation and detract from the open water nature of these types of studies. The third solution is effective but must be done frequently to avoid buildup of biomass, otherwise an unrealistic route for transfer of materials occurs. The last two solutions are realistic, overcome problems arising from high side area to volume ratios but place restrictions on the types of studies that can be carried out.

If studies focus on biota with an obvious sedentary form of existence, then side and bottom effect are desirable; e.g. in a stream channel. Aquaria are best for this type of study because increasing any dimension gives increased volume as well as the side and bottom areas with little effect on the proportionality between the two.

(b) Complexity

Biomagnification of certain contaminants could be demonstrated in a model ecosystem containing 2 or 3 trophic levels with a single species at each level. However, transfer rates between levels and concentration of residues are likely to be unrealistically high because of a forced routing of the contaminant through a single food chain. Ideally, multi-specific communities at several trophic levels would offer the interactive food webs characteristic of nature (Figure 7). If we assume that each biotic component in a system can take-up a contaminant accumulated in or eliminated from another component and that all components can take up contaminant directly from the water, then the

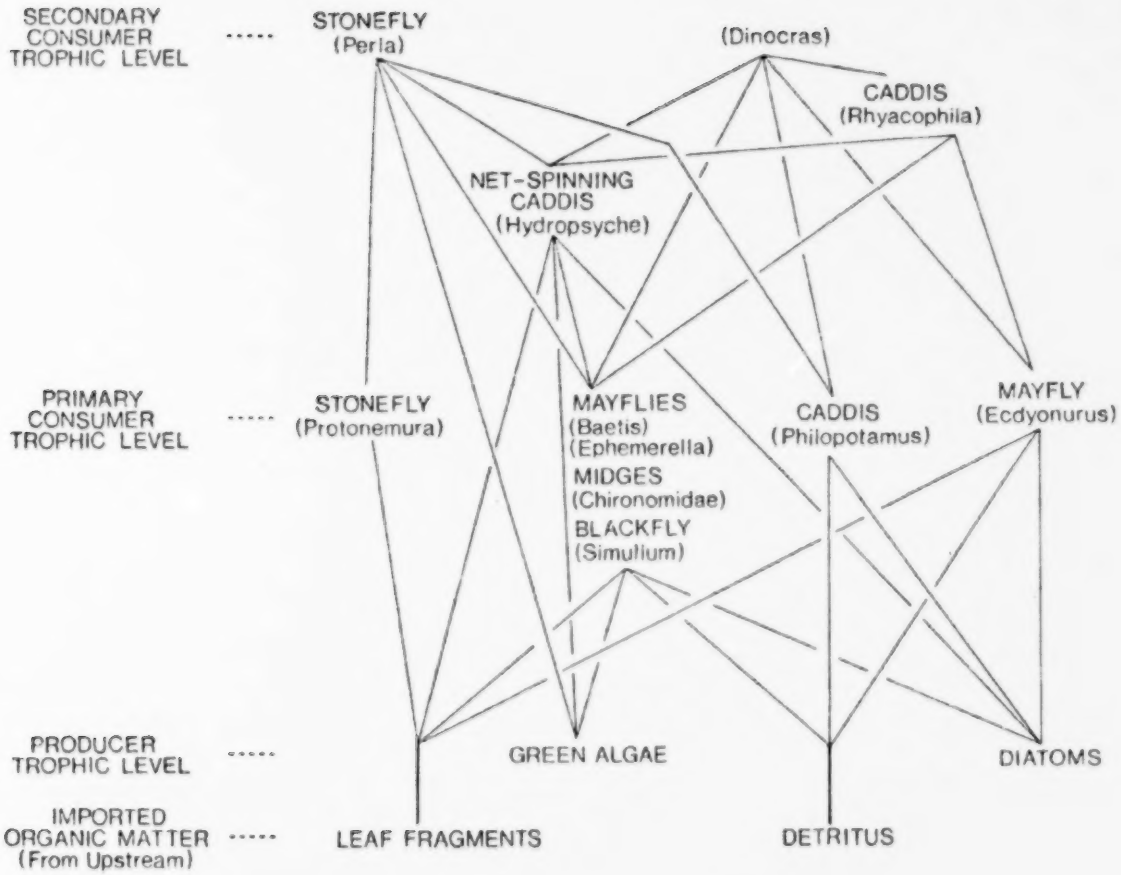


Figure 7. Interactive food web of a stream showing possible routes for contaminant transfer, independent of uptake from water (from Odum, 1959).

number of possible routes for transfer increases exponentially with the addition of each component (Fig. 8). Since some transfer routes will be favoured over others depending on community structure, the situation illustrated is even more difficult to analyze. Complexity is desirable for realism but grouping of components for study and modelling is an obvious necessity. The disadvantages of increasing complexity are that processes are more difficult to understand, the number of measurements required increases, and system size must increase to allow realistic numbers and densities of organisms at each trophic level. In other words, increased complexity gives increased realism but at an increased cost.

(c) Productivity

The movement of substances entering an ecosystem is likely to be affected by energy flow. Consequently, the ability to manipulate primary productivity of a model ecosystem is desirable. Manipulation of primary productivity is easier if only one of its forcing factors, light or nutrients is limiting.

Indoor model ecosystems permit control of both factors yet many experiments provide only low-intensity fluorescent lighting. The first experiments in the lake column simulators at GLBL carried out under fluorescent lighting only (11 K lux) showed that light intensity was a limiting factor for development of large standing crops of phytoplankton. Addition of high intensity tungsten-halogen lamps (70-100 K lux) provided the energy necessary to drive the systems. The result has been sustained higher levels of primary production and phytoplankton biomass, better replication between columns and more rapid establishment of phytoplankton communities. High intensity lighting offers a more natural situation where productivity is primarily influenced by nutrient loadings.

(d) Contaminant Additions

Toxicity of a contaminant is usually assessed in standard bioassays by continuous addition of a toxicant to maintain a concentration. Many factors affect concentrations of contaminants measured in natural systems. Therefore, it is difficult to establish the level of exposure biota are subjected to in nature. The use of a loading rate or the amount of a contaminant added per unit area or volume per unit time is probably more realistic but difficult to establish. For example, if we were interested in the effect or fate of the contaminant from a particular river on a lake we could:

1) add the total contaminant load of the river per unit time to our model ecosystem
2) divide the total load by the lake surface and add in proportion to the surface area of the model ecosystem
3) divide the total load per unit time by the area over which the contaminant is known to be distributed in the lake and add to the model ecosystem in proportion to its surface area or
4) do alternatives 2 and 3 on a volume basis.

None of the above are completely acceptable. The first

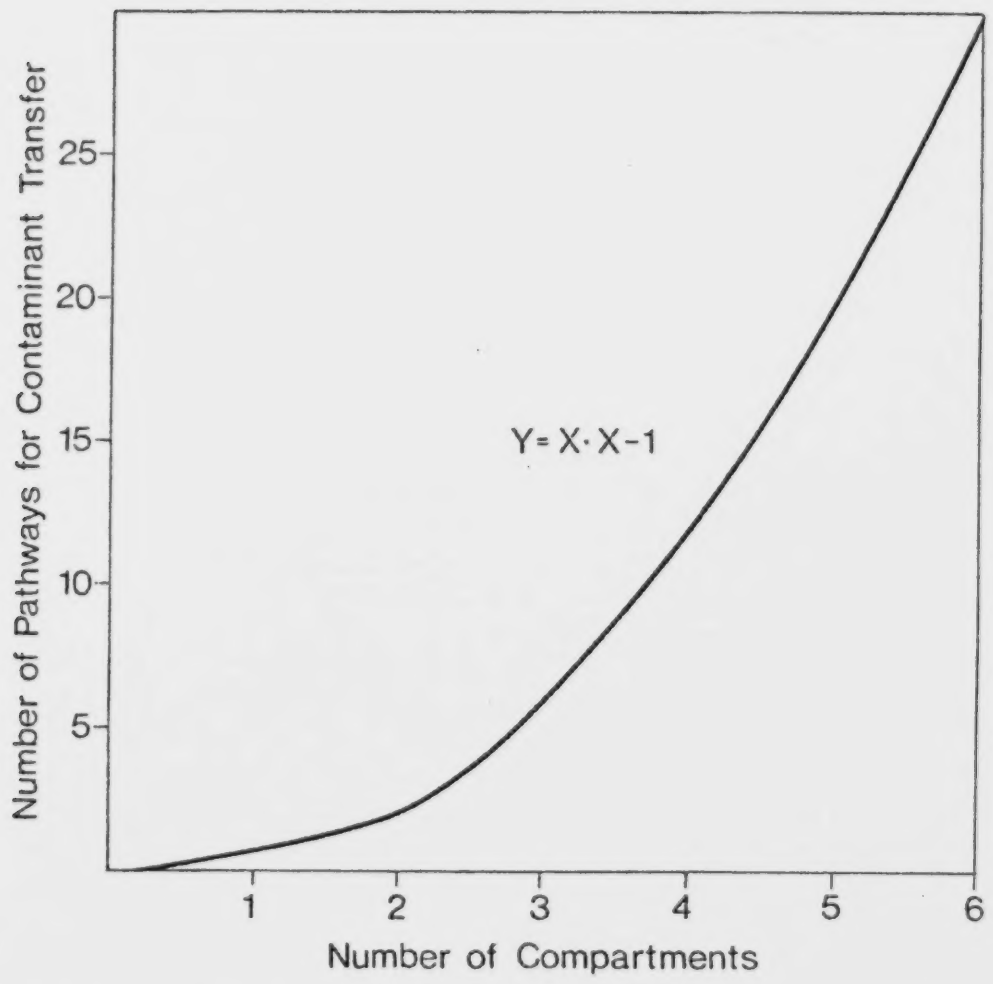


Figure 8. Exponential increase in numbers of pathways for comtaminants transfer as the number of model compartments increase (includes uptake from water).

alternative ignores differences in scale, the second assumes the impact of each river to be relevant to the whole lake and the third requires information rarely available.

A compromise is to estimate realistic concentrations and maintain them during the experiment by a suitable loading rate.

Natural modes of entry of contaminants into the model ecosystem should be employed. For example, the majority of the PCB's entering the great lakes via river inputs are not in solution but are absorbed to particles. Addition of a contaminant such as PCB's in an organic solvent or aqueous solution would be unrealistic.

(e) Flow-Through Vs Static

Static model ecosystems (those without constant addition of water) permit the population of a species to progress from the inoculum stage through a log growth phase to senility and population crash.

In flow-through systems, wash-out of plankton species occurs, the overall level of productivity may be lower, and the species rarely achieve senility but can be maintained at some relatively constant growth rate. The result is a chemostat and a steady state of productivity. Introduction of new species in the influent may also increase stability. In large static lake simulators, precipitation of organisms to the bottom provides some "wash-out" and may contribute to a steady state or dynamic equilibrium.

The cost of flow-through is lower population densities and increased difficulty in measuring performance and contamination at each trophic level due to smaller sample sizes. Dosing is more complex and mass balances more difficult to calculate. For radio-actively labelled compounds, the cost of the contaminant also becomes important because of losses via outflow.

CONCLUSIONS

Synthetic model ecosystems provide relatively cheap, fast and simple hazard assessments of environmental contaminants. However, they only provide a relative ranking of hazard due to their artificiality. Model ecosystems using natural assemblages are larger, more complex and more expensive but provide significant savings over equivalent field studies. Contaminants dynamics will be close to those observed in the field when the characteristics of the model ecosystem approach those of the field. Results are sensitive to container size, ecosystem complexity, productivity, and the way in which water and contaminant are added. Use of these systems will provide significant savings of time and money if they are used to prescreen lists of contaminants so that only the most hazardous are studied in the field.

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A SYSTEMATIC APPROACH TO AQUATIC CONTAMINANT SURVEILLANCE

by

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ABSTRACT

Craig, G. R., K. Suns,D. L. Wells, C. A. Curry and T. Lagan. 1978. Systematic approach to aquatic contaminant surveillance. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C. November 8-19, 1977. Fish. Mar. Serv. Tech. Rep. C18.

Selected fish species with limited home ranges can be used to indicate localized organic contamination. Collection of young-of-the-year age classes provides a known period of exposure and a sound historical data base. Pinpointing contaminant discharge areas by relocating bivalves from low contamination areas to appropriate sites is practical and aids in locating contaminant sources.

Fish exposed to selected industrial effluents for 48 hours will develop flavour impairment as an illustration of non-specific organic accumulation. Accumulated organics in fish exposed to treated sewage waste may be identified using an on-line biomonitoring system being designed into a municipal sewage treatment plant. This approach will provide a longer term record of the types of organics being generated which might be hazardous to public health.

A SYSTEMATIC APPROACH TO AQUATIC CONTAMINANT SURVEILLANCE

INTRODUCTION

Contaminant surveillance programs have generally been of a widespread nature designed to identify levels of contamination in biota and to determine whether human health might be affected. Monitoring programs have grown to the point where expenses and management have become difficult.

The accent of previous programs has been to establish historical information such as in the Federal-Ontario collections monitoring levels of DDT, PCB's and Hg in commercial and sports fish. Other studies have incorporated caged fish to determine the effects of current spray programs (Lockhart et al.,1977), the effects of industrial discharges on fish survival flavour impairment (Hasselrot, 1975) and changes in fish behaviour (Birtwell, 1976).

Recent developments in analytical technology and methodology during the last decade have not only allowed greater identification between specific organics (e.g. DDT vs PCB's) but also identification of a wider variety of compounds as a result of combining mass spectrometry with gas chromatography (GC-MS).

As a consequence of this increased analytical capability, a change in contaminant surveillance philosophy must be instituted. Future problems must be identified before they become a matter of record and the process should take the form of an active searching pattern for contaminants. The searching should begin with a general survey becoming more specific as contaminants are identified. When sources are located, appropriate abatement measures can be incorporated.

Incorporation of biological sampling into this surveillance approach avoids the complex issue of weighing the chemical and physical interactions of contaminants in the aquatic environment and allows greater focus on the biologically important compounds. An added feature of biological sampling includes the retention of organic compounds by biota which otherwise would be seen as fluctuating concentrations in the water column.

This presentation promotes a systematic approach in contaminant surveillance progressing from general to specific monitoring. Examples of completed work indicate how evaluations of localized areas, specific sites and discharges as well as on-line biomonitoring can be practically implemented. The underlying assumption is that regulatory agencies can provide the sophisticated analytical support for such a program.

REGIONAL EVALUATIONS

The first step in identifying contaminated areas involves selection of a representative biological organism which has a localized home range

and can provide a homogeneous sample.

The general lack of sample homogeneity in fish residue investigations has complicated data evaluations, especially when station to station comparisons are necessary to determine geographic distributions of contaminants. The general requirements for a homogeneous sample can be expressed in terms of relative uniformities of age, size and fat content. It is also essential that a life stage with a restricted home range be selected.

In order to achieve a degree of sample standardization, young-of-the-year spottails (Notropis hudsonius) were collected at nine collection sites on the Lower Great Lakes during the fall of 1975 (Fig. 1). The spottail shiner was chosen because of its general abundance in the study area, ease of collection with unsophisticated fishing gear and its restricted home range (W.B. Scott, personal communication). Distribution of the spottail shiner in the Great Lakes is extensive and it is considered a common forage species (Emery, 1976).

Preliminary data from Ontario inland lake surveys of 1977 suggests that young yellow perch (Perca flavescens) populations are equally useful for bio-accumulation investigations.

Fish Collection and Analysis

A 20 meter seine with 0.6 cm mesh was used for all collections. Fish were captured in 1-2 meter depths during the first two weeks of September 1975. Fish were measured, wrapped in hexane rinsed aluminum foil as ten fish composites and frozen in the field.

Identification and quantitative analyses for PCB, HCB, BHC, lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, thiodan, chlordane, as well as DDT and its metabolites were done by gas chromatographic techniques. Lipid contents of fish were determined by hexane extraction and expressed as a percentage of total body weight (M.O.E.).

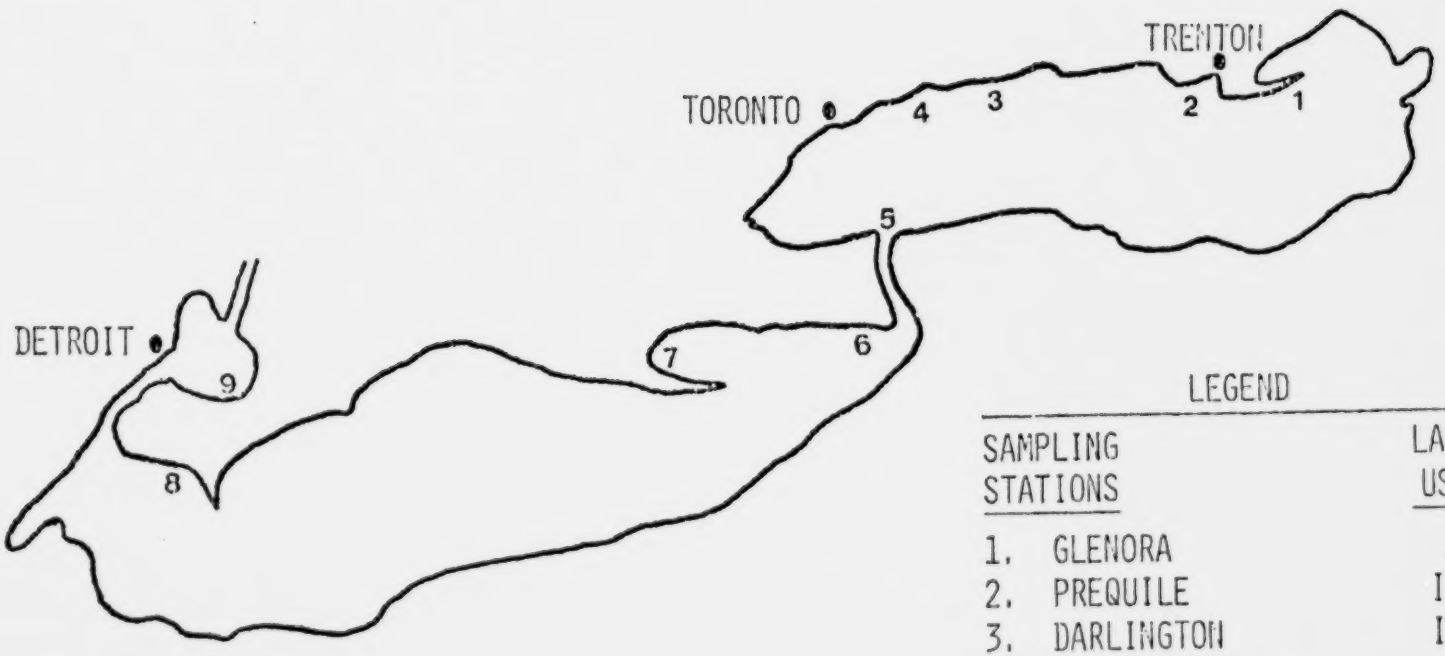
Results and Discussions

All samples analyzed contained PCB and DDT residues. Heptachlor epoxide was present in all but Frenchman's Bay and Point Pelee samples, while dieldrin, endrin and chlordane were found in Presquile collections only (Table 1).

The highest DDT and metabolite residues were found in Niagara-on-the-Lake and Port Rowan locations (Figure 2). Former land-use practices at both locations were considered to be conducive to DDT accumulations in soils (Harris and Miles, 1975). While extensive use of DDT in the tobacco industry and its impact on the biota at Port Rowan has been documented (Frank et al., 1974; Harris and Miles, 1975), proper evaluation of the DDT residue load carried by the Niagara River is lacking.

Significant differences in PCB concentrations were found in the young-of-the-year spottails when comparing residues in fish from different stations (Figure 3).

Figure 1: Spottail shiner collection sites on Lake Ontario, Lake Erie and Lake St. Clair.



LEGEND

SAMPLING STATIONS		LAND USE
1.	GLENORA	A
2.	PREQUILE	IA
3.	DARLINGTON	IA
4.	FRENCHMANS BAY	A
5.	NIAGARA	IA
6.	PORT COLBOURNE	A
7.	PORT ROWAN	A
8.	POINT PELEE	IA
9.	TREMBLAY CREEK	IA

A - AGRICULTURAL
I - INDUSTRIAL

Station No.		Number of Analysis	Mean Fish Length (T.L.) (mm)	% Fat Content	PCB	ΣDDT	Heptachlor Epoxide	Dieldrin	Endrin	Chlordane α	Chlordane γ
1	Clarence	5	64 ± 4	2.9 ± 0.3	111 ± 27	41 ± 7	1 ± 0				
2	Prescôtle	5	60 ± 4	2.7 ± 0.2	520 ± 91	77 ± 12	2 ± 2	1 ± 2	1 ± 0		1 ± 1
3	Darlington	5	64 ± 4	4.5 ± 0.8	420 ± 116	91 ± 29	1 ± 1				
4	Frenchman's Bay	5	52 ± 5	2.2 ± 0.3	200 ± 35	46 ± 10					
5	Niagara-on-the-Lake	5	56 ± 4	2.3 ± 0.3	690 ± 195	244 ± 52	1 ± 1				
6	Port Colborne	5	61 ± 3	1.2 ± 0.3	82 ± 29	32 ± 23	1 ± 1				
7	Port Rowan	5	65 ± 4	2.1 ± 0.6	59 ± 29	128 ± 65	1 ± 1				
8	Point Pelee	5	63 ± 3	1.8 ± 0.2	844 ± 403	92 ± 22					
9	Ire Clay Creek	4	65 ± 5	3.4 ± 1.9	275 ± 207	81 ± 54	2 ± 1				

Table 1: Organochlorine contaminant residues (ng/g) in young-of-the-year spottail shiners from Lakes Ontario, Erie and St. Clair.

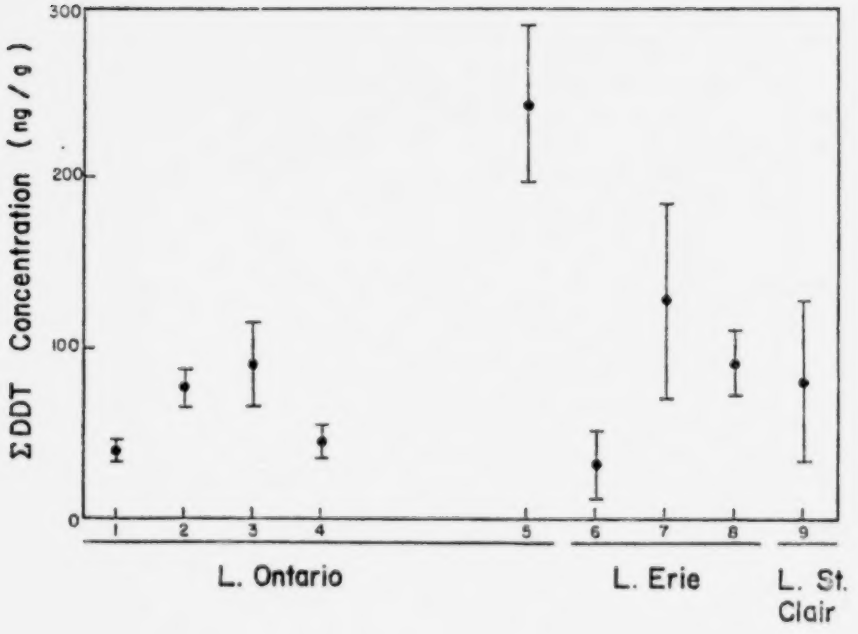


Figure 2: Mean DDT residues in spottail shiners, with 95% confidence limits, from lakes Ontario, Erie and St. Clair.

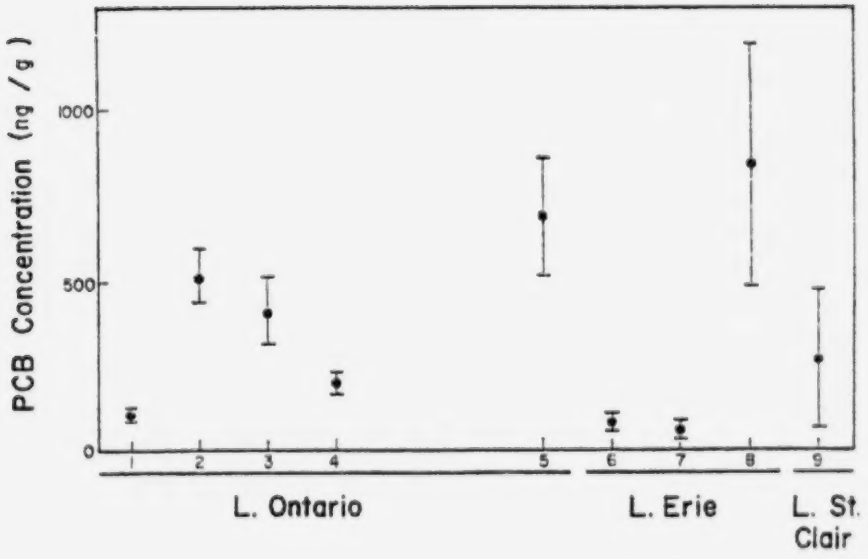


Figure 3: Mean PCB residue levels in spottail shiners, with 95% confidence limits, from Lakes Ontario, Erie and St. Clair.

The highest PCB levels in fish were found in Presquile, Darlington, Niagara-on-the-Lake and Point Pelee collections. All four sites are located near industrial areas: Presquile being associated with Trenton-Belleville, Darlington with Oshawa, Niagara-on-the-Lake with Niagara Falls-Buffalo, and Point Pelee with the Detroit-Windsor complex. It should be noted that the highest PCB residues in fish were obtained from areas affected by the Niagara and Detroit River discharges, both major contributors of PCB's to Lakes Erie and Ontario(R.L.Thomas, pers. communication). Fish from the predominantly rural areas of Glenora, Port Colborne and Port Rowan had the lowest PCB residue levels (Figure 3). Intermediate PCB residue levels were found in Frenchman's Bay and Tremblay Creek samples. The former site is primarily agricultural with some urban development, while Tremblay Creek is affected by industrial, agricultural and urban discharges.

Fish sizes, age and fat contents were relatively uniform for all collections which provided sample homogeneity. Lipid content versus residue concentration relationships were not observed ($P > 0.05$; $r = 0.15$) consequently contaminant level differences were considered to reflect local conditions.

This study indicated that the young-of-the-year spottail shiner responds quantitatively to land use activities. This fact combined with the relatively sedentary nature of the species make it a useful organism for contaminant trend monitoring.

SITE EVALUATIONS

Identification of local areas of contamination through fish collections described previously, leads to more specific contaminant assessment at selected sites. The organism chosen for this stage of the investigation was the freshwater clam (Elliptio complanata).

The sedentary nature of bivalves and their continuous filtering of surrounding waters makes them an ideal organism for site specific contaminant bioaccumulation studies. Numerous researchers have used bivalves successfully in uptake experiments involving metals and organics (Smith et al., 1975; Maki and Johnson, 1976; Bedford et al., 1968; Petrocelli et al, 1977; Boelens and Rumsey, 1972). These investigations have shown the initial uptake of contaminants to be quite rapid with levels magnifying those of the water by several times. Miller et al. (1966) found mussels accumulated diazinon in 24 hours at concentrations twice that of the water. In cases where water analyses showed non-detectable contaminant levels, bivalve tissue has indicated elevated contaminant levels (Bedford et al., 1968).

The advantages of transferring the freshwater clam (Elliptio complanata), from low level contaminant areas to those of suspected contamination are described and identification of contaminant sources is illustrated.

Clam Collection and Transfer

Clams were collected from Balsam Lake, Ontario and their lengths and weights were recorded. Controls were shucked, weighed and tissue was wrapped in hexane rinsed aluminum foil and frozen for later analysis.

Ten clams were placed in each cage. Cages were constructed of galvanized wire with the dimensions 22 x 21 x 15 cm. Each cage was identified and secured in the mouth of the Humber River. Cages were placed on the river bottom (≈1m).

Two cages were retrieved at 8, 18 and 29 days from the initial date of exposure.

Prior to analysis clams were again counted, measured and weighed. The flesh was weighed and wrapped in hexane rinsed aluminum foil.

Extraction of pesticides and PCBs was done by azeotrophic distillation using the solvent benzene. Extraction was followed by Florisil clean-up and gas chromatrographic analysis (M.O.E.).

Results and Discussion

Clams from Balsam Lake contained low to non-detectable levels of DDT, PCB, dieldrin and chlordane (Figure 4, 5 and 6).

Clams placed in the Humber River accumulated all of the above pesticides, except chlordane, within the first eight days of exposure (Figures 4 and 5). Elevated levels of α and β chlordane did not appear until 18 days of exposure (Figure 5). With the exception of dieldrin, there was no further significant accumulation of organics after 18 days.

The above results indicate that significant accumulation of organics by clams may occur within eight days and as much as 18 days. It is also evident that the accumulation rates of organics may differ as illustrated by the greater exposure time required for chlordane to reach elevated levels. Alternatively, it is possible that chlordane was not present until after the eighth day.

The caging technique used in this study provided easy placing and relocation of the clams. Although the clams were transferred from their natural lake habitat to a river system they did survive the duration of the 29 day exposure period. Their apparent hardiness after such treatment and lack of artificial feeding adds further strength to their practicability as a monitoring tool. Caged clams may be situated in areas inappropriate for caged fish or where fish capture methods are inadequate, above and below discharge pipes to determine the nature and extent of discharge or at river mouths to delineate between the river and lake impact on biota.

Caging techniques in general can confirm suspected organic contaminant sources and the use of clams as opposed to fish, further simplifies this approach.

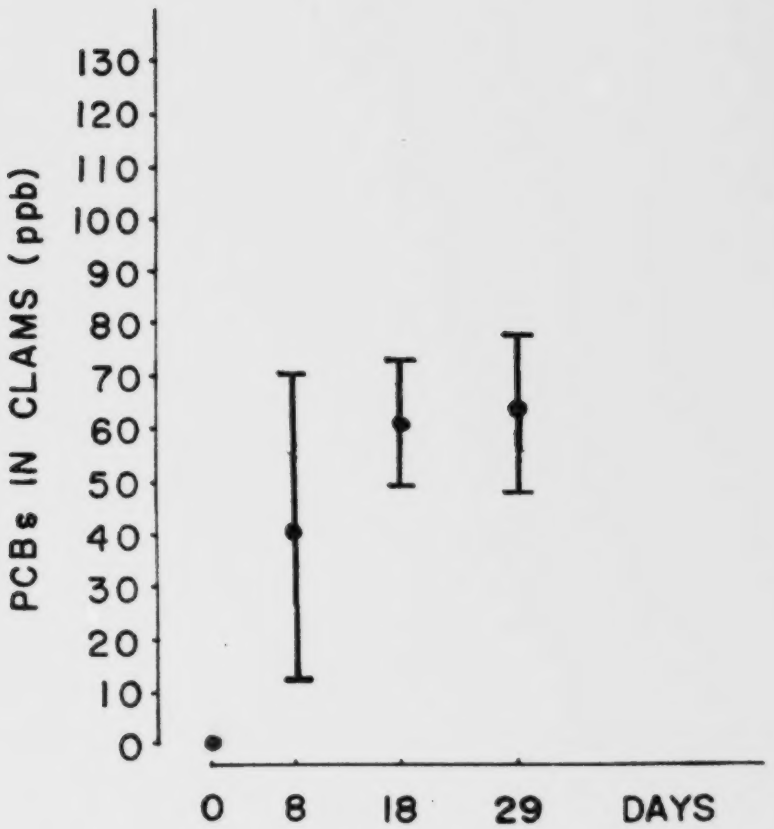


Figure 4: Accumulation of PCB's by clams during 29 days exposure in the Humber River (means ± 95% confidence limits).

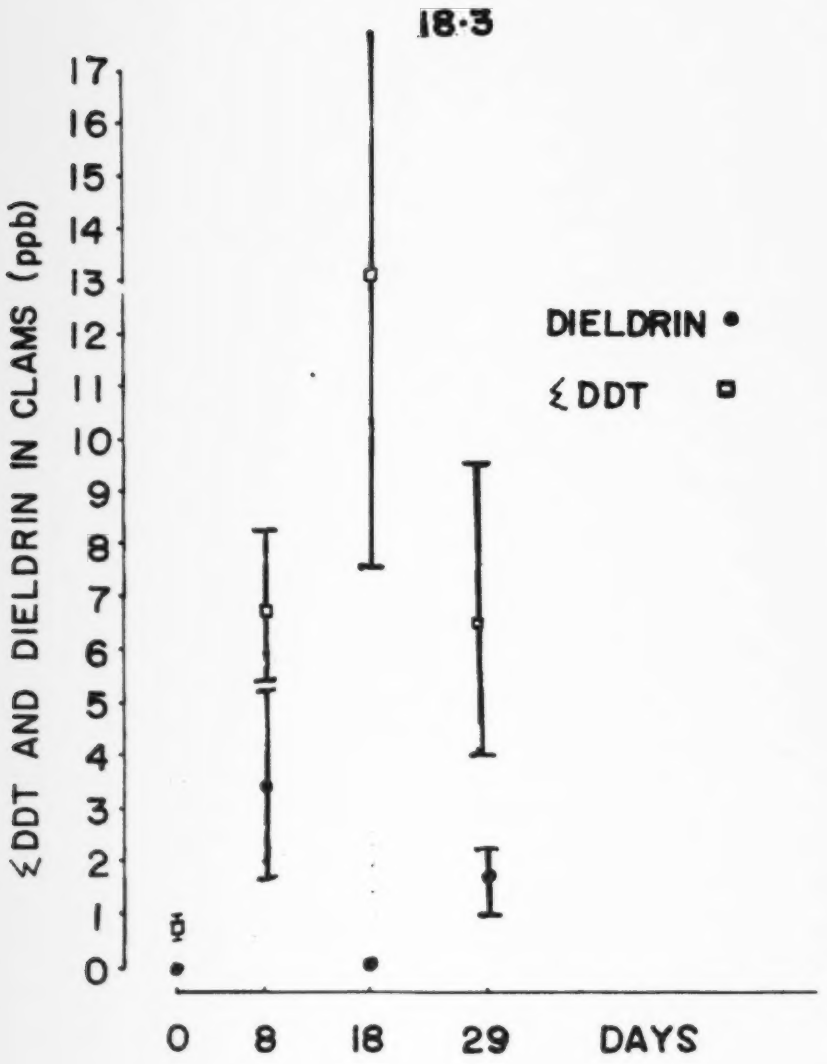


Figure 5: Accumulation of ΣDDT and Dieldrin by clams during 29 days exposure in the Humber River (means ± 95% confidence limits).

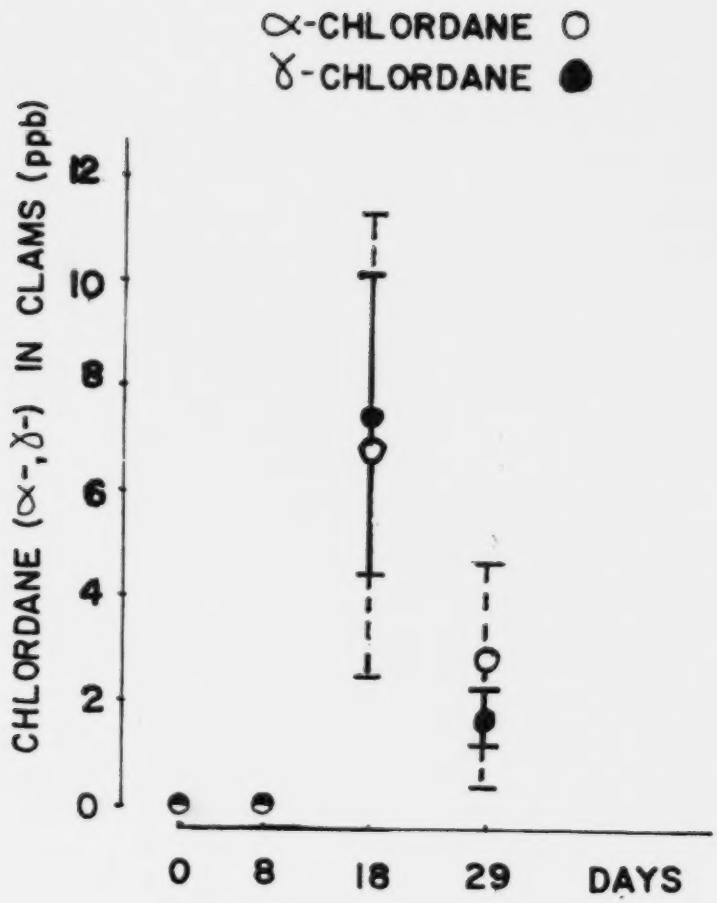


Figure 6: Accumulation of α chlordane and γ chlordane by clams after 29 days exposure in the Humber River (mean ± 95% confidence limits).

DISCHARGE EVALUATIONS

Once contaminant sources have been narrowed to specific discharges the organics can be quantified by collecting samples of effluent for more specific testing. Although previous examples have dealt with pesticides and PCB's as representative organics, industrially related compounds can also be identified in fish using GC-MS techniques.

This example describes the evaluation of selected chemical industrial effluents using lethality, fish flavour and GC-MS analysis. The waste effluents studied originated from the following processes.

Plant #1 - production of benzene, toluene, xylene, polyvinyl chloride.

Plant #2 - activated sludge plant treating oily water wastes from a petroleum refinery.

Plant #3 - composite sewer draining wastes from styrene, ammonia, styrene-butadiene latex and ethylene production units.

Plant #4 - production of toluene diisocyanate.

Effluent Collection and Fish Exposure

An effluent sample of about 800 gallons was transported to Toronto and stored at 4°C for 24-48 hours. Static 96 hour LC50's were determined to establish the potency of the waste prior to continuous flow exposure of the fish. Four 250g rainbow trout were exposed for 48 hours in a 225 ℓ aquaria to about 3.5 standing volumes of each concentration per 24 hours. Exposure concentrations were 100, 50, 10, 5% v/v plus a control for all effluents except that from plant #1 which was tested at half the above values (eg 50, 25, 5 and 2.5% v/v). Following exposure the fish were sacrificed, weighed, measured, identified and frozen until required for the taste test the next week.

Taste Test

Six persons comprised the taste test panel. Each panelist received coded samples of cooked fish at least one of which was an unidentified control. The flavour rating system consisted of a four point scale where "1" indicated no difference from the known control and "4" indicating strong difference from the known control. Wilcoxon's modification of Friedman's rank test compared results from effluent exposed fish with that of the blind controls. More detailed test protocols followed ASTM methodology (ASTM, 1968).

Fish not incorporated in the taste test were stored for GC-MS analysis as were effluent samples used in the study.

Results and Discussion

Only Plant #1 effluent produced lethality (96 hr LC_{50} = 95%) in rainbow trout.

Preliminary data indicate that fish tainting occurred in non-lethal effluent concentrations and organics identified in the effluent were accumulated by the fish (Table 2). It must be noted that organic levels were measured in the 100% effluent from samples taken 24 hours after the beginning of the test and only fish exposed to 100% waste have been analyzed. Indications are that the organics identified were concentrated in the fish up to 1000 times that measured in the effluent (Table 2).

It is evident that complex organics can be accumulated by fish in a short time and that taste test evaluations can indicate non-specific organic accumulation. Additionally, it is apparent that the rates of accumulation among organics differ.

The analytical support required by this testing procedure is extensive and is probably limited to government and university laboratories.

ON-LINE BIOMONITORING

The logistics of transporting large volumes of effluent to a central laboratory are avoided by the incorporation of an in-line exposure facility at the point of discharge. The inherent difficulty of obtaining a "representative sample" using grab methods is also avoided.

On-line systems have been established, in conjunction with industrial discharge, by Cairns et al. (1973) to monitor activity and breathing rates in sunfish. Alterations in behaviour indicated changes in effluent quality. Events were not only of a short term nature but also required specialized recording equipment and technical personnel at each site. The technical complexities of such an operation can be eliminated if the fish exposed on-site are analyzed for organic accumulation in a central laboratory. This simplified approach increases the geographical range of installations and efficiently utilizes the expensive analytical equipment.

The Guelph sewage treatment plant is currently including a bio-monitoring room in its expanded facilities to be completed in 1979. The initial program will include direct exposure of fish to undiluted, unchlorinated final effluent with no temperature control. More rigorous testing of chlorinated effluent with temperature control will be incorporated at a later date when appropriate funds are acquired to support the additional cost. The future system is designed with a high degree of automation so that the facility can be operated by unskilled personnel.

TABLE 2: Accumulation of organics from effluent which produced tainting of fish flesh.

Plant No.	Tainting Concentration % v/v	Organic Compound	Concentrations in Fish exposed to 100% Effluent	Concentrations in 100% Effluent
1	≥ 5%	Benzene	88 ppb	49 ppb
		Toluene	1113 ppb	12.4 ppb
		Xylene (S)	375 ppb	Detected
		Styrene	310 ppb	N.D.*
		Cumene	175 ppb	37 ppb
		Trimethyl Benzene	300 ppb	N.D.*
		Diethyl Benzene	1200 ppb	12 ppb
		Naphthalene	250 ppm	4 ppm
		Methyl Naphthalene	1150 ppm	N.D.*
2	≥ 10%	$C Cl_4$	1 ppb	32 ppb
		Toluene	100 ppb	N.D.*
		Xylene (S)	10 ppb	N.D.*
		Styrene	6 ppb	N.D.*
3	≥ 10%	$C Cl_4$	N.D.	25 ppb
		Benzene	.6 ppm	N.D.*
		Toluene	7.3 ppm	1.2 ppb
		Xylene (S)	6.1 ppm	6.3 ppb
		Styrene	17 ppm	N.D.*
		Trimethyl Benzene	0.3 ppm	4.1 ppb
		Diethyl Benzene	9 ppm	10 ppb
		Napthalene	0.048 ppm	N.D.*
		Methyl Napthalnene	0.15 ppm	N.D.*
4	≥ 50%	$CH Cl_3$	Detected	7 ppb
		$C Cl_4$	100 ppb	44 ppb
		$C_2 Cl_4$	120 ppb	19 ppb
		Toluene	30 ppb	N.D.*
		Xylene (S)	29 ppb	70 pptr
		Styrene	38 ppb	N.D.*
		Trimethyl Benzene	19 ppb	N.D.*
		Dichloro Benzene	4300 ppb	6 ppb
		Trichloro Benzene	600 ppb	N.D.*

* Non-detected

Proposed Facility and Exposure Design

Three exposure tanks, two holding tanks and one display indicator tank comprise the exposure facility (Figure 7). Fish in the exposure tanks will be subjected to chlorinated and unchlorinated effluent and dilution water (control). Effluents will be diluted to allow long term survival of fish. Chlorine and chlorine residuals will be removed from chlorinated effluent and control water by the addition of sodium thiosulphate. The display tanks will receive dilution water unless ammonia or conductivity levels indicate a slug discharge. When slugs are sensed in either effluent the flow will be diverted to the display tank to register possible lethality. Cost estimates for equipment and services are $25,000 with annual operational costs estimated at $5,000.

Fish species for the exposure and display tanks have not been selected but will provide sufficient tissue for analysis.

Fish will be identified by tagging to enable a number of groups to be exposed at one time. This will allow periods of exposure to overlap thereby providing more of a continuous exposure program.

Discussion

The previously described studies illustrate the ability of fish to accumulate organic contaminants. Consequently, a biomonitoring system can indicate present trends in contaminants. Compounds that are repeatedly identified will provide direction for abatement programs since many organics can be related to specific industries or industrial operations.

The chlorination of organics in sewage treatment plants during the disinfection process is well documented (Jolly 1973; Painter 1973). Industries currently use sewage treatment plants as a means of avoiding waste treatment and the variety of organics present in sewage plant effluents indicates that many of these industrial wastes cannot be adequately treated by these municipal facilities. The discharge of organics is of particular concern when municipalities draw supply water downstream of the sewage treatment plant.

The variety of contaminants that can enter sewage treatment plants, which are generally owned and operated by local or provincial governments, could provide good justification for the installation of biomonitoring systems. Such installations would benefit the interests of public health through consequent pollution abatement programs and would provide an example for industry to encourage improved monitoring of contaminants discharged in their effluents.

CONCLUSION

The biomonitoring examples cited to detect organic contaminants are not novel, having been developed and reported in the literature over the

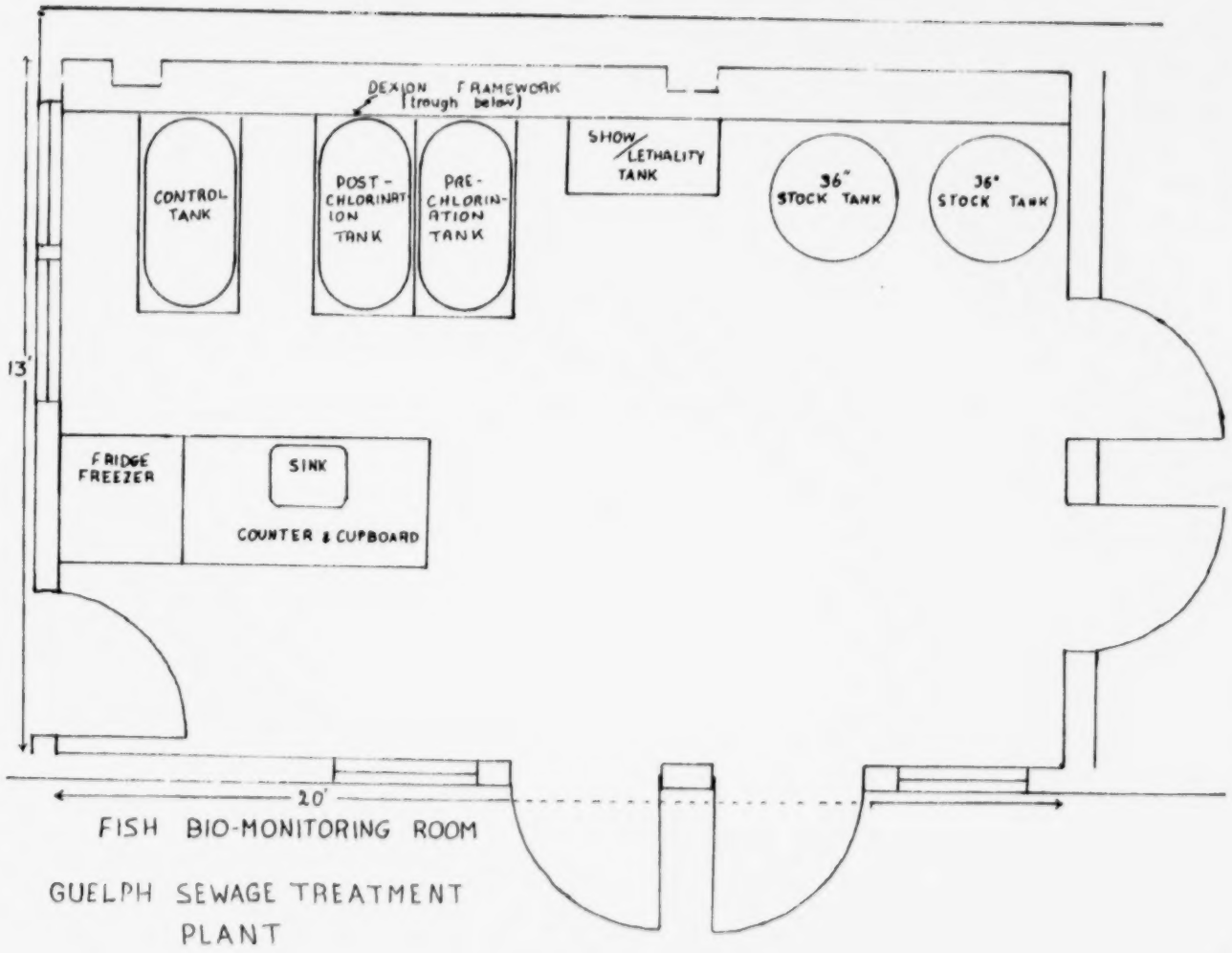


Figure 7: Biomonitoring room floor plan to be incorporated in Guelph Sewage Plant expansion.

last twenty years or more. However, recent advances in analytical technology have increased the scope of these monitoring methods. Instead of being limited to defining specific contaminant concerns, a variety of methods can be incorporated to identify unsuspected contaminant problems and provide direction for abatement programs.

This change in philosophical approach, as additional techniques are incorporated, will pose new problems in methodology. The selection of the appropriate organism, the accumulation rates of different organics in those organisms, the effects of age, feeding, physiological and behavioural responses will affect the interpretation of results. The relationships between biological accumulation of organics and physical and chemical characteristics of water, suspended solids and sediments will provide quantitative interpretation. These goals will have to be better defined as questions become increasingly demanding.

The biomonitoring examples used in this presentation illustrates that existing foundations can be used to actively identify contaminant problems utilizing general, and progressing to specific assessment techniques. The ultimate goal in all of these programs should not be establishment of historical record but abatement of pollution practices.

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TOXICITY OF PRIMARY SEWAGE EFFLUENT TO MARINE INDICATOR ORGANISMS IN PUGET SOUND

by

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Stober, Q. J., P. A. Dinnel, M. A. Wert, and R. E. Nakatani, 1978. Toxicity of Primary Sewage Effluent to Marine Indicator Organisms in Puget Sound. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

ABSTRACT

The toxicity of primary chlorinated sewage effluent was determined for five marine organisms by utilizing continuous flow acute and chronic bioassays with 0.5 to 60% v/v sewage dilutions in ambient seawater. Acute bioassays tested the effects of reduced salinity, temperature, sewage filtration, dechlorination with sulfur dioxide, and ammonia removal with clinoptilolite resin. Eight-week chronic bioassays were used to assess the uptake of the trace metals copper and zinc by shiner perch exposed to sublethal concentrations of sewage. Histopathological examinations of fish from acute and chronic bioassays were conducted.

The mean 96-h LC50 values for shiner perch and English sole in chlorinated effluent were 16.1 and 15.4% v/v, respectively. The 96-h LC50 for coonstripe shrimp ranged from 15 to 20% v/v. The Pacific staghorn sculpin and shore crab were least sensitive, with a 96-h LC50 of 30% v/v and a 120-h LC50 of 50% v/v, respectively. Reduction of salinity, dissolved oxygen, and filtration of the effluent caused minimal change in the toxicity to either shiner perch or English sole. An increase in temperature to 18.5 C (Δt = 10 C) lowered the LC50 from 18 to 11% for shiner perch. Shiner perch showed a consistent sensitivity between year classes while zero age English sole were twice as sensitive. Dechlorination and ammonia removal decreased toxicity of chlorinated sewage to shiner perch and English sole by approximately a factor of two.

Histopathologic analysis of shiner perch in chronic bioassays showed signs of focal edema and separation of epithelial cells from underlying vascular tissue at 0.5% chlorinated effluent. Whole body trace metal analysis of shiner perch showed no bioaccumulation of Cu, but body burden of Zn increased. Zn may have been accumulated from the food. However, as time of exposure to effluent increased, whole body burden of Zn decreased. The maximum acceptable concentration of chlorinated effluent which may safely be discharged to Puget Sound was 0.5% v/v.

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INTRODUCTION

The Puget Sound Interim Studies Program sponsored by Seattle METRO included the bioassay of West Point sewage effluent using marine organisms indigenous to the outfall area. The Interim Studies conducted near the West Point outfall and in central Puget Sound provided ecological data needed to support an environmental impact assessment. To aid interpretation of the field data, a bioassay program was initiated to investigate the real or potential effects of the effluent under semi-controlled environmental conditions. Bioassays were designed to determine the effects of some of the physical and chemical components of the effluent on shiner perch (Cymatogaster aggregata), English sole (Parophrys vetulus), Pacific staghorn sculpin (Leptocottus armatus), coonstripe shrimp (Pandalus danae), and shore crabs (Hemigrapsus nudus). The volume of effluent discharged through the West Point outfall averaged from 284 x 10^3 to 341 x 10^3 m^3/day. Release was through an offshore diffuser at a depth of 73 m.

A mobile marine bioassay laboratory which utilized flowing ambient seawater and sewage effluent was constructed at the West Point Sewage Treatment Plant. The quality of the effluent was tested as it came from the plant and further modified by additional treatment to remove ammonia, chlorine, and/or particulates. Acute and chronic bioassays of the untreated and treated effluent were tested. Analysis of toxic components in the sewage effluent, verification of concentrations of toxicants tested, and determination of tissue accumulations of trace metals in chronically exposed organisms were made throughout the duration of the tests conducted over one annual cycle.

MATERIALS AND METHODS

Laboratory

A mobile marine laboratory was designed and constructed in 1975 at the West Point Treatment Plant. The seawater distribution, heating and temperature control systems, and the monitoring equipment were similar to those described by Stober (1972) and Stober, et al. (1977 a).

The laboratory was a 3.0 x 16.8 m converted mobile home, containing a 3.0 x 12.2 m wet laboratory, and a 3.0 x 4.6 m dry laboratory-office. Adjacent facilities included a 2.4 x 7.3 m cargo container housing treatment equipment for both seawater and sewage, a prefabricated metal shed housing a fish behavior lab, and several exterior fish holding tanks.

Seawater was continuously pumped at a rate of 12.6 ℓ/sec from an intake located off the north side of West Point at a depth of 7.6 m at lower low water. Primary treated, chlorinated sewage effluent was piped to a head tank at the laboratory for distribution.

A set of treatment chambers was constructed to remove ammonia and/or chlorine, two of the principal toxicants in the sewage (Fig. 1). Peagravel filters were utilized for seawater and effluent. A series of four treatment chambers contained ion-exchange resin (minus 4 mesh clinoptilolite) for ammonia removal. Two chambers were injected with sulfur dioxide (SO_2) gas through a Fisher & Porter gas flow metering valve to dechlorinate the effluent. The SO_2 columns were filled with 2.5 cm porcelain saddles to insure adequate mixing and contact time with the SO_2. The treated effluent was pumped to the laboratory and behavior shed for distribution to the bioassay tanks.

Each set of filter or treatment chambers (except SO_2) was a dual system such that one side was available to supply filtered (or treated) effluent to the lab while the other side was backflushed or recharged. Seawater was used to backflush the seawater filters and recharge the ion-exchange resin. The effluent filters were backflushed with freshwater.

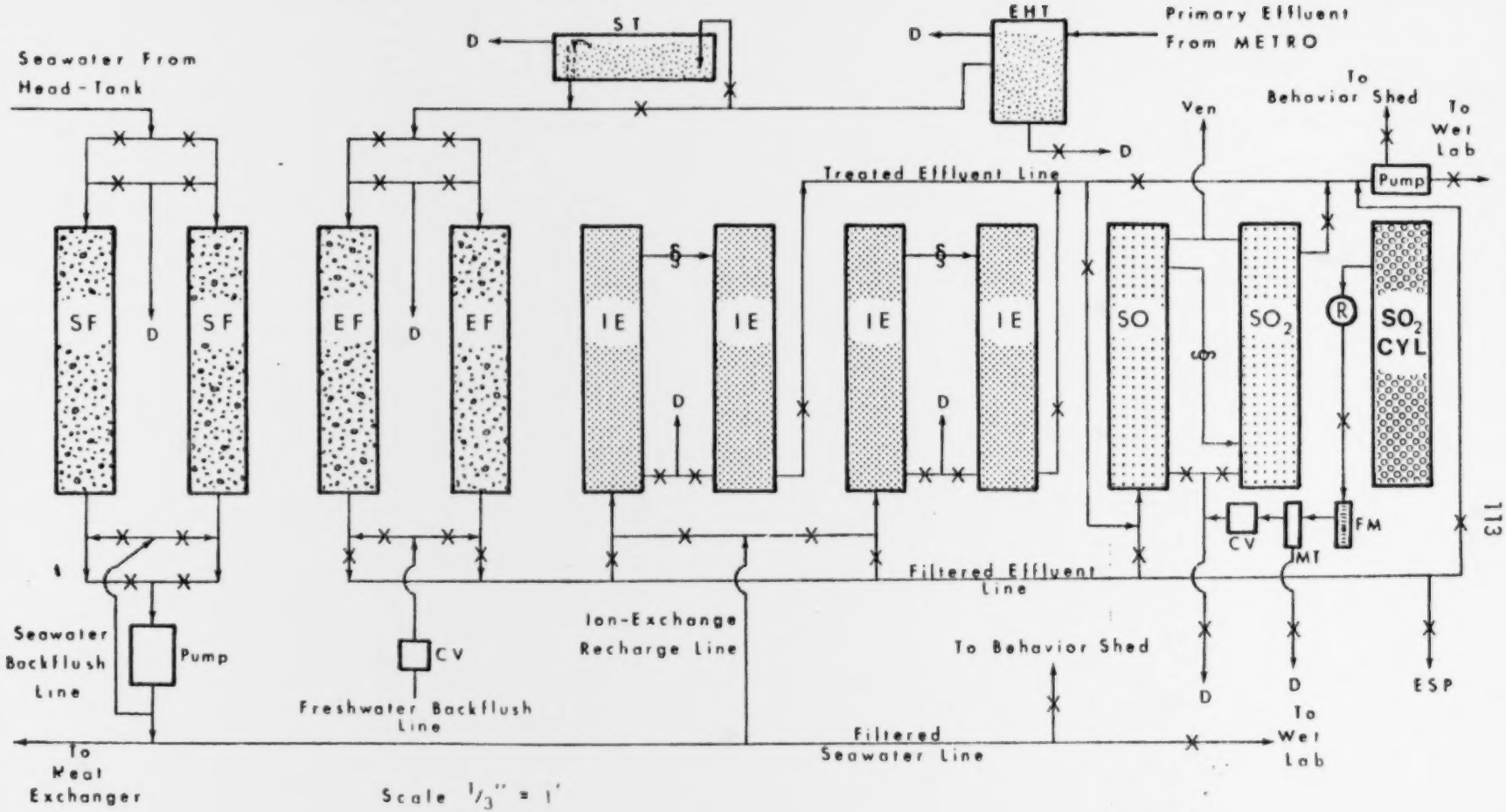
Water Quality Monitoring

The methodology and equipment used for monitoring the physical-chemical parameters of the bioassays are summarized in Table 1. Temperature, salinity and dissolved oxygen were measured directly in each tank with as little disturbance to the test animals as possible. Samples for turbidity, pH, total residual chlorine, ammonia, and sulfur dioxide were collected. Acute bioassays were sampled at 24-hr intervals, except during ammonia removal or dechlorination when the frequency was increased to a schedule identical to the mortality monitoring.

Additional physical-chemical sampling data for the effluent was obtained from 24-hr composite samples analyzed by METRO water quality laboratories. METRO sampling and analytical methodology are summarized in Table 2 and include daily information on rainfall, sewage flow rate, temperature, pH, DO, BOD, COD, total suspended solids, volatile suspended solids, and settleable solids. Weekly data was obtained on ammonia as nitrogen (NH_3-N), total nitrogen, phosphate as phosphorus (PO_4-P), and grease. Total residual chlorine (TRC) was monitored continuously. Trace metals cadmium, chromium, copper, mercury, nickel, lead, zinc, and hexavalent chromium were measured in daily 24-hr composite samples.

Test Animals

English sole, Pacific staghorn sculpin and shiner perch were collected by beach seine from the north beach at West Point. Additional shiner perch were seined from Kopachuck State Park in south Puget Sound. Coonstripe shrimp were captured by otter trawl off West Point. Shore crabs were



CV – Check Valve
D – Drain
EF – Effluent Filter Column
EHT – Effluent Head-Tank
ESP – Effluent Sampling Point
FM – Gas Flow Meter
IE – Ion-Exchange Column
MT – Moisture Trap
R – Gas Pressure Regulator
SF – Seawater Filter Column
SO_2 – SO_2 Dechlorination Column
SO_2 CYL – SO_2 Gas Supply Cylinder
ST – Effluent Settling Tank
X – PVC Single-Union Valve
§ – PVC Union

Figure 1. Flow diagram of seawater and sewage effluent filter chambers and effluent ammonia removal (by ion-exchange) and dechlorination (by sulfur dioxide) chambers.

Table 1. Equipment and methodology of water chemistry analysis for West Point Sewage Effluent (WPE) acute and chronic bioassays.

Test		Equipment	Type Measurement	Standard	Detection† Limits	Units
Temperature	1.	C^{o} Thermometer	Direct		S = 0.1 R = 0-100	Degrees Centigrade (^{o}C)
	*2.	ARA electronic thermometer with scanner and recorder	Direct	C^{o} thermometer	S = 0.25 R = 0-55	
Salinity	1.	Hydrometer	Conversion of reading by tables		S = .01	Parts per Thousand ($^{o}/oo$)
	*2.	Portable Beckman salinometer and probe	Direct	Hydrometer	S = .01 R = 0-99	
Dissolved Oxygen (DO)	1.	DO bottle and titration equipment	Winkler Titration	0.025 N PAO	S = .01	Parts per Million (ppm)
	*2.	YSI DO meter and probe	Direct, after calibration in sat. air	Winkler Titration	R = 0-20 S = 0.05	
Turbidity		Hach kit	Direct, based on light transmission	Distilled water blank	R = 0-500 S = 1	Jackson Turbidity Units (JTU)
pH		Orion specific ion meter with pH and reference probes	Direct, after calibration with pH standards	Orion pH standard solutions	R = 0-14 S = 0.05	Standard pH Units
Total Residual Chlorine (TRC)		Wallace and Tiernan amperometric titrator	Back titration	Distilled water blank	R = 0.05-10 S = 0.05	Parts per Million (ppm)
Ammonia (NH_3-N)		Orion specific ion meter with gas sensing electrode	Direct, after calibration with 2 standards	Orion ammonium chloride standard solution	R = 0.017-17,000 S = variable	Parts per Million (ppm)
Sulphur Dioxide (SO_2)		Orion specific ion meter with gas sensing electrode	Direct, after calibration with 2 standards	Orion sulphur dioxide standard solution	R = 0.1-1000 S = variable	Parts per Million (ppm)

† R = Range; S = Sensitivity

*Normal monitoring method

Table 2. Summary of methodology used by METRO laboratories for chemical analysis of West Point Sewage Effluent.

Test	Method	Units
Rainfall	Rain gauge at West Point Treatment Plant	inches
Average Flow Rate	Flowmeter	million gallons/day (MGPD)
Temperature	Thermometer	^{o}C
Dissolved Oxygen (DO)	DO meter and probe	ppm
pH	Continuous monitor pH meter and probe	pH units
Biological Oxygen Demand (BOD)	Standard Methods, p. 474*	mg/l
Chemical Oxygen Demand (COD)	Dichromate reflux method, Standard Methods, p. 495*	mg/l
Total Residual Chlorine (TRC)	Continuous monitor amperometric titrator	ppm
Ammonia (NH_3 as N)	Phenolhypochlorite method	ppm
Total Nitrogen (N)	Kjeldahl Digestion	ppm
Phosphate (PO_4 as P)	Vanadomolybdophosphoric acid colorimetric method	ppm
Grease	Freon 113 extractable	ppm
Suspended Solids	Standard Methods, p. 537*	mg/l
Settleable Solids	Standard Methods, p. 539*	mg/l
Volatile Suspended Solids	Standard Methods, p. 538*	mg/l
Hexavalent Chromium	1-5 diphenolhydrocarbohydrazide direct wet test method	mg/l
Other Trace Metals	Atomic absorbtion spectrophotometry	mg/l

* Standard Methods for the Examination of Water and Wastewater. 13th ed., American Public Health Association, New York, NY. (1971).

collected by hand from a rocky intertidal beach north of West Point.

Test animals were transferred from the capture site to holding tanks in seawater filled plastic containers. Oxygen was added when transport time exceeded 30 minutes. All test animals were held and acclimated in large circular or rectangular fiberglass tanks supplied with a constant flow of ambient seawater for at least one week prior to testing. Test animals were fasted three days prior to use in acute bioassays; but fish used in chronic bioassays were fed throughout acclimation and the entire test period.

Bioassays

Acute (96-hr) and chronic (8-week) bioassays were conducted with effluent using two calibrated siphon tube continuous flow diluter systems (Fig. 2). Flow rates were controlled by the tube diameter and fine-tuned by vertical movement of the siphon to maintain the flow rate ±1% of that desired. All siphon tube flow rates were set at a minimum of 0.5 ℓ/min to minimize clogging.

The test tanks were 53 ℓ glass aquariums with a flow-through volume limited to 40 ℓ. Flow rates ranged from 1.0 to 1.4 ℓ/min as recommended (Anon., 1975). Organism loading rates (10/tank) varied with species, but never exceeded 0.15 g/ℓ of the test tank volume. All tanks, funnels, and plexiglass tubes were scrubbed with detergent and freshwater and rinsed with concentrated hydrochloric acid between bioassays. The glass siphon tubes were replaced for each bioassay.

A series of dilutions of primary chlorinated effluent (0, 1, 5, 10, 25, and 50% v/v) were used to determine the approximate LC50. The effects of reduced dissolved oxygen were tested by aeration of dilutions of 0, 20, 40, and 60% v/v effluent in seawater. The reduction of salinity was tested at 0, 20, 40, and 60% v/v effluent to determine the effects on test animals. Also increased temperature on the effluent toxicity was tested at 8.5 C (ambient), 12.5 and 18.5 C at dilutions of 0, 20, 40, and 60% v/v effluent. The potential effects of filtering effluent were also checked.

Life stage mortality differences of age zero and age 1+ English sole and shiner perch were tested with serial dilutions of effluent in seawater. Both species were used to test the difference in toxicity between chlorinated and dechlorinated effluent. SO_2 residual was maintained between 1 and 4 ppm. Chlorinated effluent was tested against effluent with ammonia removed by passage through clinoptilolite resin chambers.

Mortality was monitored at 1 to 2 hr intervals for the first 6 hrs, 2 hr intervals for the next 6 hrs, 4 hr intervals up to 48 hrs, and 24 hr intervals for the following 48 hrs. Geometric mean survival times (Brown, et al., 1967; Sprague, 1969) and 96-h LC50 concentrations (Anon., 1975) for each species under each set of conditions were calculated.

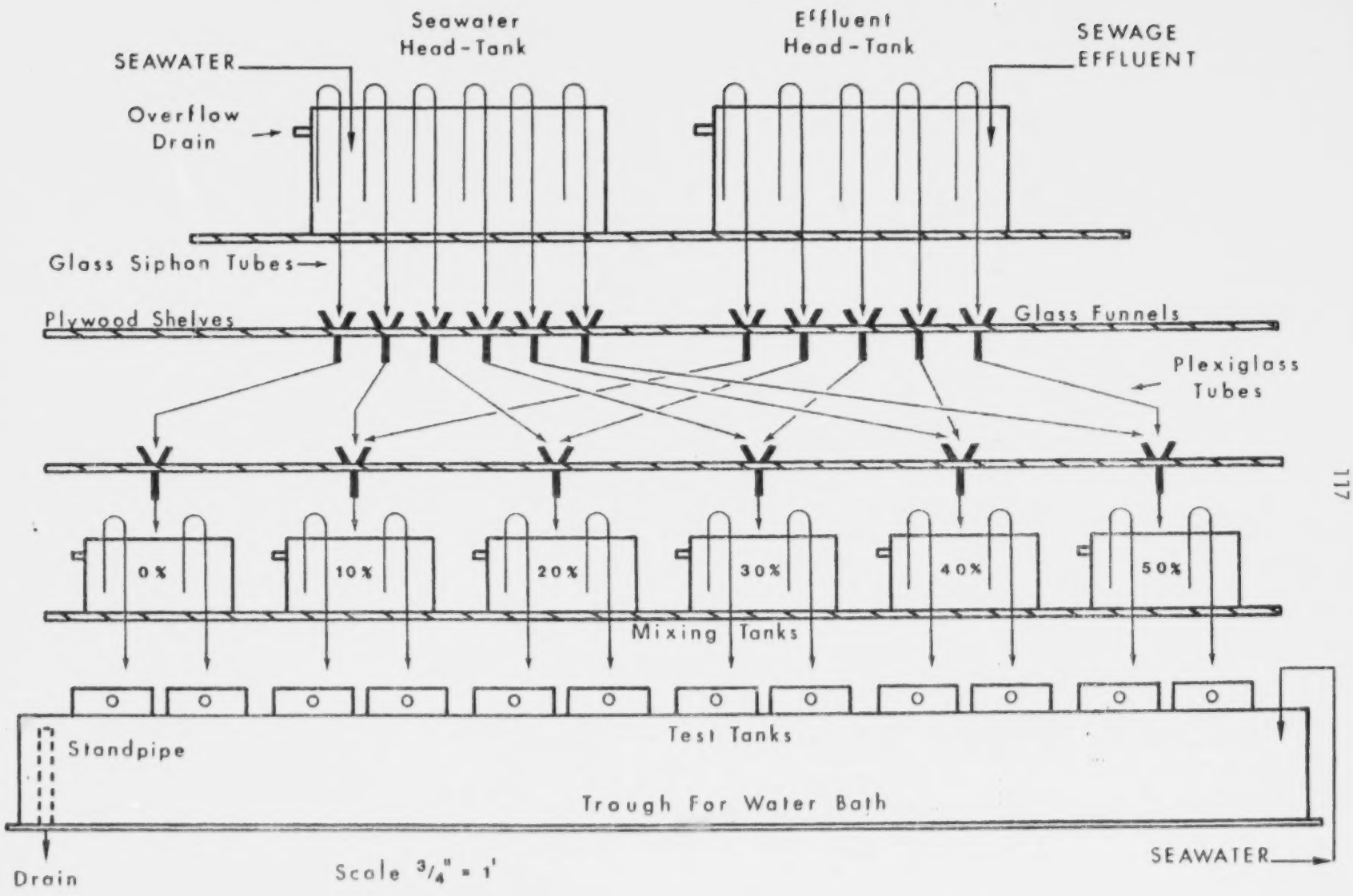


Figure 2. Diagram of sewage effluent diluter system. Percentage values are percent effluent in seawater v/v. Rubber drain hoses from overflow drains to floor drains are not shown.

Shiner perch and English sole were tested in three 8-week continuous flow chronic bioassays conducted from February to November, 1976. Effluent concentrations of 0, 0.5, 1, 2.5, and 5% v/v were tested. Flow rates to the test aquariums were 1.5 ℓ/min, sufficient for a 95% replacement in approximately 1 hour (Sprague, 1969). The daily flow into test aquariums was at least 15 ℓ/g of organism/day.

Throughout the course of the experiment, fish were fed commercial frozen paneid shrimp or moist food pellets prepared at the University of Washington fish hatchery. Fish food and unexposed test fish were frozen for trace metal analysis. Fish were starved for 4 days prior to sampling to eliminate residual food from the gut. Fish samples were wet ashed in nitric and perchloric acids (Smith, 1953) with trace metal concentrations measured by atomic absorption spectrophotometer.

Histopathology

Samples of gill tissue were collected from shiner perch and English sole exposed to acute and chronic concentrations of effluent and to controls exposed to ambient seawater. Gill tissue was excised immediately after death and placed in Bouin's fixative for 24 hrs. Samples were then dehydrated in 30 and 50% ethyl alcohol for 4 hr intervals and stored in 70% alcohol. Later laboratory preparation included dehydration, clearing, and embedding in paraffin. Tissues were cut to 5 microns, stained in hematoxylin and eosin and mounted on glass slides for examination.

RESULTS

Seasonal Changes

Seasonal change in the ambient temperature, salinity, and dissolved oxygen of the marine receiving waters was apparent during the annual period over which tests were conducted (Fig. 3). The minimum and maximum mean temperature ranged from 7.7 C in March to 12.6 C in August. Mean salinity was lowest at 26.9 ppt during winter when maximum freshwater runoff occurred. Maximum salinity of 31.2 ppt was observed in September. Mean dissolved oxygen was lowest in summer (6.5 ppm) and fall (7.2 ppm). Similar fluctuations of DO and temperature occurred in the effluent (Fig. 4). Mean dissolved oxygen remained at less than 1 ppm during summer but fluctuated widely to 3.2 ppm during winter storm events. Mean effluent temperature ranged from 11.8 C in April to 19.5 C in August. The temperature of the effluent averaged 4.5 C above the winter minimum ambient and 7.1 C above the summer maximum ambient temperature. The seasonal changes in the quality of the effluent and the receiving waters were partially integrated into toxicity determinations by conducting repetitive bioassays throughout one annual cycle.

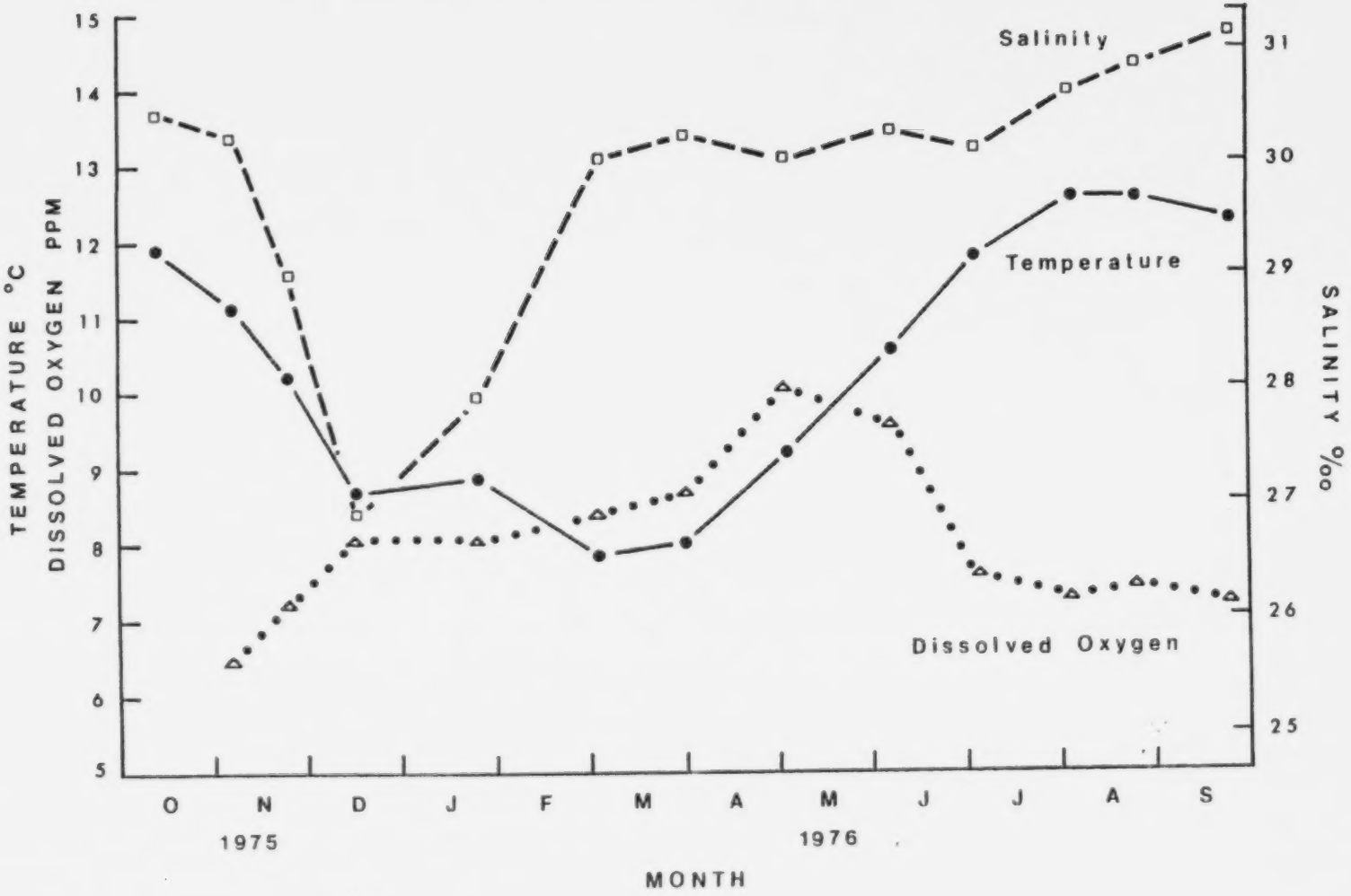


Figure 3. Mean ambient seawater temperature, salinity, and dissolved oxygen as measured in the seawater head-tank of the proportional diluter.

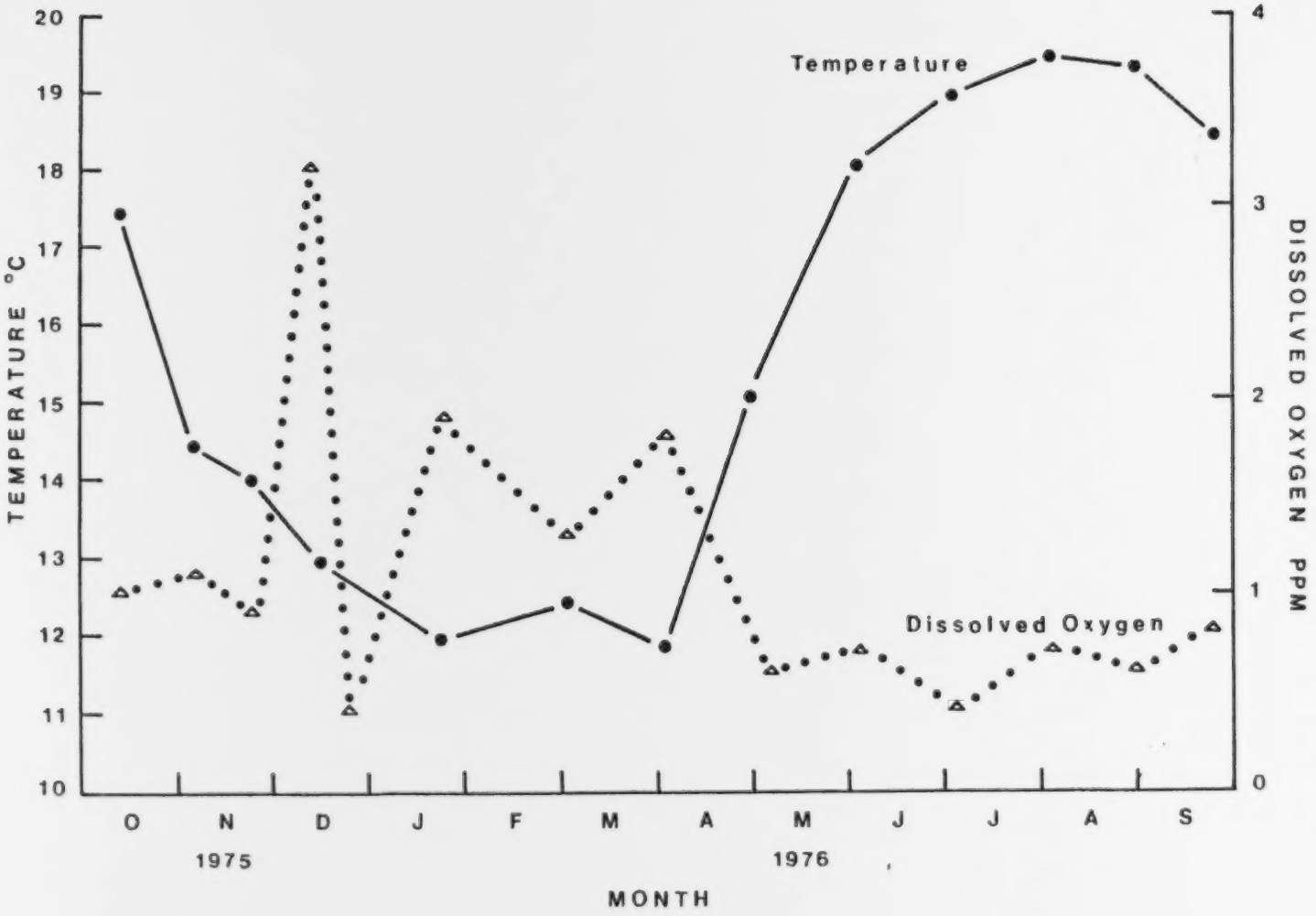


Figure 4. Mean West Point effluent temperature and dissolved oxygen as measured in the effluent head-tank of the proportional diluter.

The determination of the toxicity of sewage using fish bioassays was complicated due to the ever changing concentrations of both known and unknown toxicants. The potential for confounding the experimental design by reduced DO and salinity was specifically tested. A series of bioassays conducted with unaerated and aerated effluent indicated 96-h LC50 values for English sole were 15% v/v for unaerated and 16% v/v for aerated effluent. The 96-h LC50 values for shiner perch were 18% v/v for unaerated and 19% v/v for aerated effluent. Aeration increased the average DO about 1 ppm at 20% and up to 2 ppm at 60% effluent when compared to similar unaerated concentrations. Survival was generally longer in aerated dilutions but final LC50 values were essentially equal. The potential effects of reduced salinity on the marine organisms tested in dilutions up to 60% v/v freshwater did not indicate stress or mortality in English sole or shiner perch.

Species Sensitivity

Bioassays conducted on juvenile English sole, shiner perch, and coonstripe shrimp indicated about equal sensitivity to chlorinated effluent with estimated 96-h LC50 values between 15 and 20% v/v. Staghorn sculpin were more tolerant with a 96-h LC50 of about 30% v/v. Shore crabs were most tolerant with a 120-h LC50 of approximately 50% v/v. Shiner perch and English sole were selected as primary species for further bioassay tests based on sensitivity and availability.

Shiner perch and English sole are common inshore inhabitants of the Pacific Coast from Southern California to Southern Alaska (Hart, 1973). Both are common inhabitants of Puget Sound and are relatively abundant in the vicinity of the West Point Sewage outfall (Miller, et al., 1977).

Thermal Effects

The effect of increased seawater temperature on the toxicity of effluent to shiner perch was tested at 8.5 (ambient), 13.5, and 18.5 C with 96-h LC50 values at 18, 18, and 11% v/v effluent, respectively. The increase in toxicity at 18.5 C may have been confounded by an observed increase in total residual chlorine and a slight decrease in dissolved oxygen concentrations. Synergistic effects were suggested, however, determination of thermal effects alone will require additional testing.

Life Stage Comparisons

The sensitivity of age zero and 1+ English sole and shiner perch exposed side by side to equal effluent dilutions was determined. Age zero English sole (average length = 28.6± 6.6 mm) were tested against the age 1+ English sole (average length = 109.8 ± 16.6 mm). The age zero group proved more sensitive than the 1^+ group, with 96-h LC50 values of 8 and 16% v/v effluent, respectively.

Similar comparisons were made on age zero and 1+ shiner perch with respective mean lengths of 49.4 ± 3.4 mm and 124.4 ± 1.5 mm. The tolerance was generally the same for age zero and 1+ with 96-h LC50 values of 14 and 15% v/v effluent, respectively. The consistent sensitivity of shiner perch throughout its life cycle further indicates the value of this species as a test animal.

Filtered Effluent

Since filtration of the effluent was necessary prior to dechlorination or ammonia removal, bioassays were conducted to determine if filtration reduced toxicity. A small reduction in the toxicity was indicated by 96-h LC50 values for shiner perch in filtered and unfiltered effluent of 21 and 19% v/v, respectively. The small reduction in toxicity of filtered effluent correlated closely with decreases in turbidity and total residual chlorine concentrations.

Dechlorination

Bioassays were conducted with English sole and shiner perch to determine the toxicity of effluent dechlorinated with gaseous SO_2. Simultaneous dilutions of chlorinated and filtered effluent dechlorinated with SO_2 yielded 96-h LC50 values of 14 and 32% v/v, respectively for shiner perch (Fig. 5). In each case, the concentration of dechlorinated effluent which could be tolerated by 50% of the test fish was approximately double the amount of chlorinated effluent.

Ammonia Removal

Clinoptilolite resin was utilized to effectively reduce the ammonia levels of the effluent by more than 95%. Coincidental reduction of the turbidity occurred as well as removal of the total residual chlorine by 95%. Simultaneous dilutions of chlorinated effluent and effluent treated to remove the ammonia were tested. English sole yielded 96-h LC50 values of 14% v/v chlorinated effluent and 45% v/v effluent with ammonia removed. 96-hr LC50 values for shiner perch were 12% v/v chlorinated effluent and 26% v/v effluent with ammonia removed.

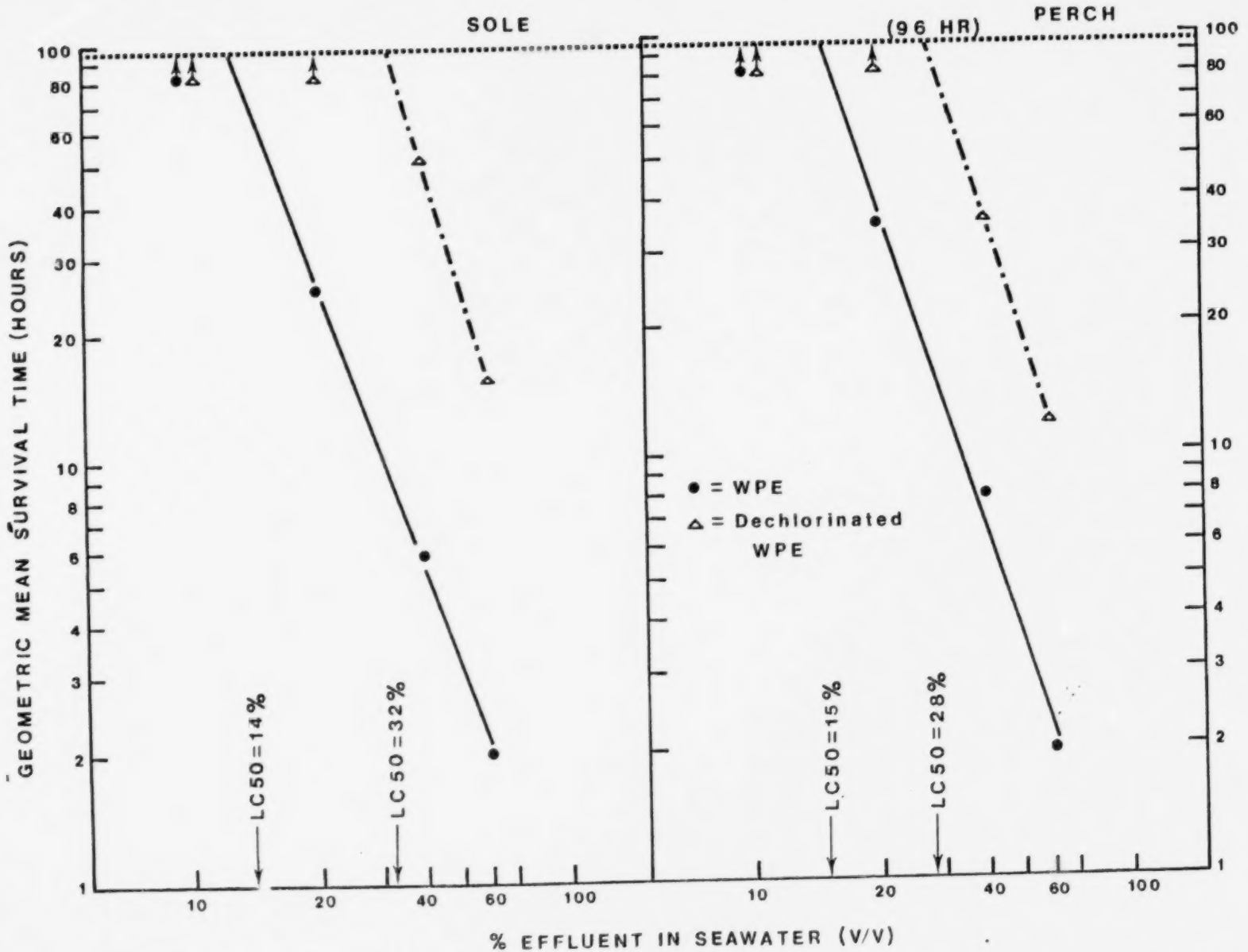


Figure 5. Determination of English sole and shiner perch LC50 in dilutions of West Point Effluent (WPE) and WPE dechlorinated with sulfur dioxide.

The ammonia nitrogen concentration in the effluent was inversely correlated with the average effluent flow (Pearson $r^2 = 0.84$), which was directly related to rainfall and stormwater runoff (Pearson $r^2 = 0.75$). The mean monthly rainfall was closely related to the mean monthly effluent flow, while ammonia increased during the summer months due to a reduction in rainfall and runoff (Fig. 6).

Excessive Mortality

Unusual fish mortality was observed during two bioassays. A bioassay testing ammonia removal with English sole was abruptly terminated at 40 hrs when all but three fish were suddenly killed in all effluent dilutions (10-60% v/v). While cause of mortality remains somewhat speculative, the METRO 24-hr composite sample for mercury and the maximum concentration for TRC showed unusually high values of 0.0037 ppm and 2.65 ppm, respectively. Mercury was substantially higher than the effluent monthly average of 0.0006 ± 0.0007 ppm. It appears that a slug of mercury was received at the treatment plant. The increase in TRC may have resulted due to chemical interference with the continuous chlorine monitor. However, chlorine was probably not the primary toxicant since fish in the effluent with ammonia and chlorine removed suffered mortality similar to those in untreated effluent.

Shiner perch in another bioassay suffered a similar fate. Unusual behavior was observed in dilutions of 10% chlorinated and 20% dechlorinated effluent, while early mortality occurred in 20% chlorinated and 40% dechlorinated effluent dilutions. Simultaneous hourly grab samples for trace metal analysis by METRO indicated that one or two slug discharges of chromium and copper were received at the treatment plant. These slugs coincided closely with the peculiar fish behavior and subsequent mortality. A coincident peak of TRC between 2.0 and 3.4 ppm lasting about 3 hrs occurred along with the slugs of heavy metals. The primary toxicant was probably not chlorine since fish mortality or abnormal behavior occurred in both chlorinated and dechlorinated effluent dilutions.

Chronic Bioassays

Shiner perch were exposed to continuous flow sublethal dilutions of chlorinated primary effluent for 8 weeks. Experimental dilutions bracketed the theoretical dilution of the effluent (140:1) at the submarine diffuser site (Bendiner and Ewart, 1976). Samples of fish were removed at day 0, and 2, 4, 6, and 8 weeks exposure for later analysis to assess the change in copper and zinc body burden through time. Copper and zinc were selected for analysis because these trace metals consistently occurred in highest concentrations in the effluent.

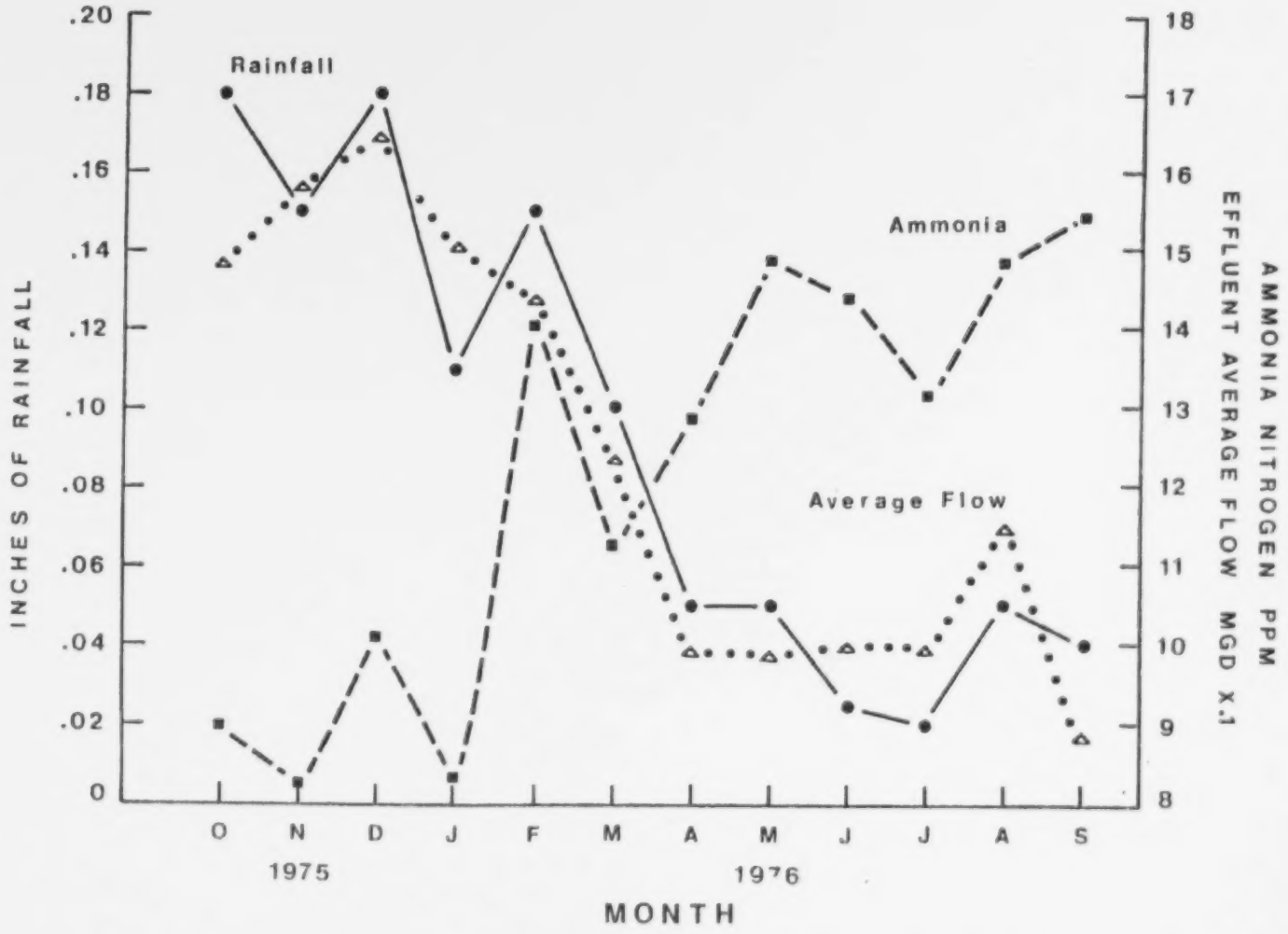


Figure 1. Average rainfall, effluent flow, and effluent ammonia nitrogen per month from October 1975 to September 1976, as measured by METRO Water Quality Staff at West Point.

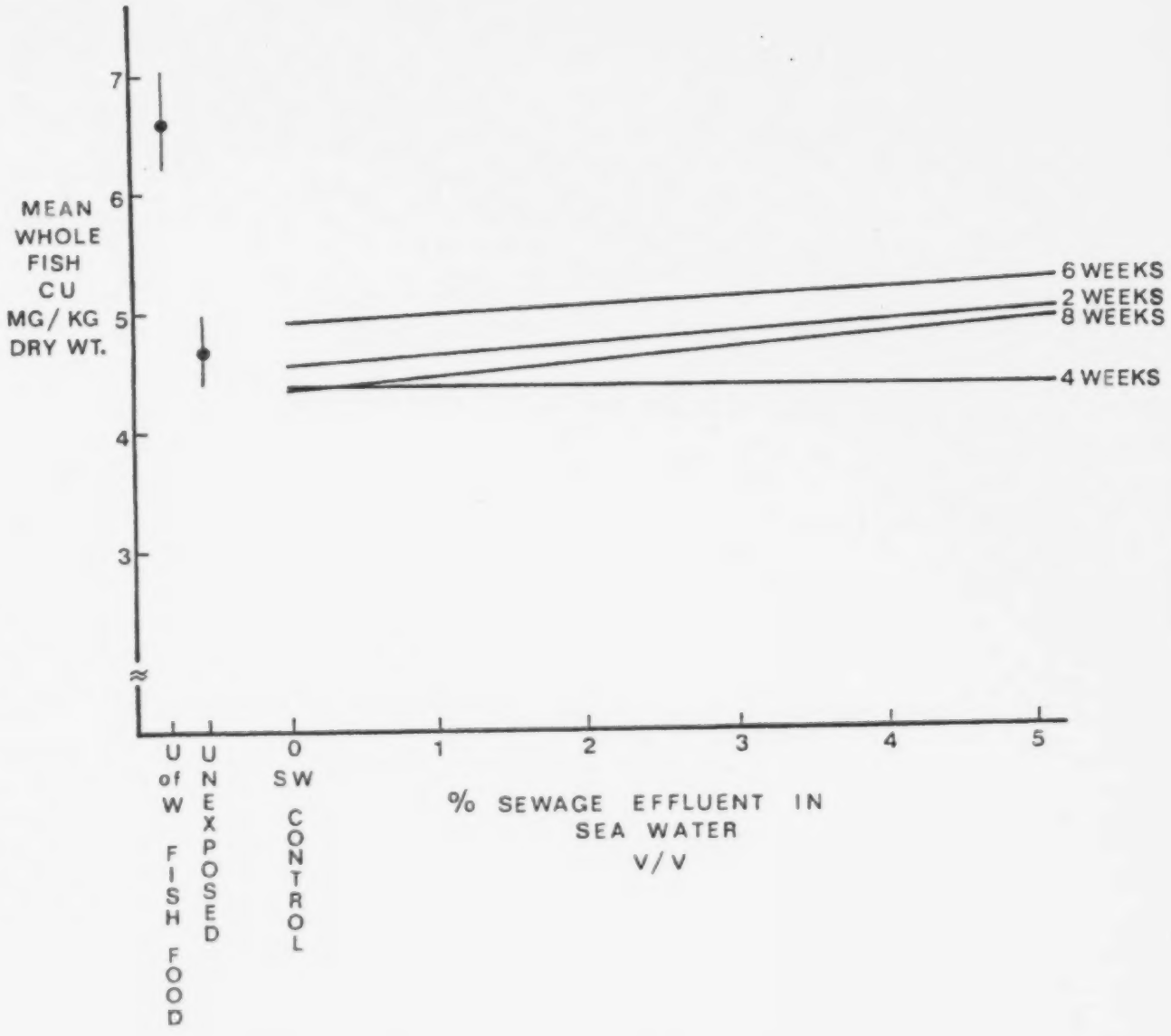


Figure 7. Summary of least square lines of mean whole fish copper content vs. exposure to dilutions of primary treated sewage effluent for 2, 4, 6, and 8 weeks.

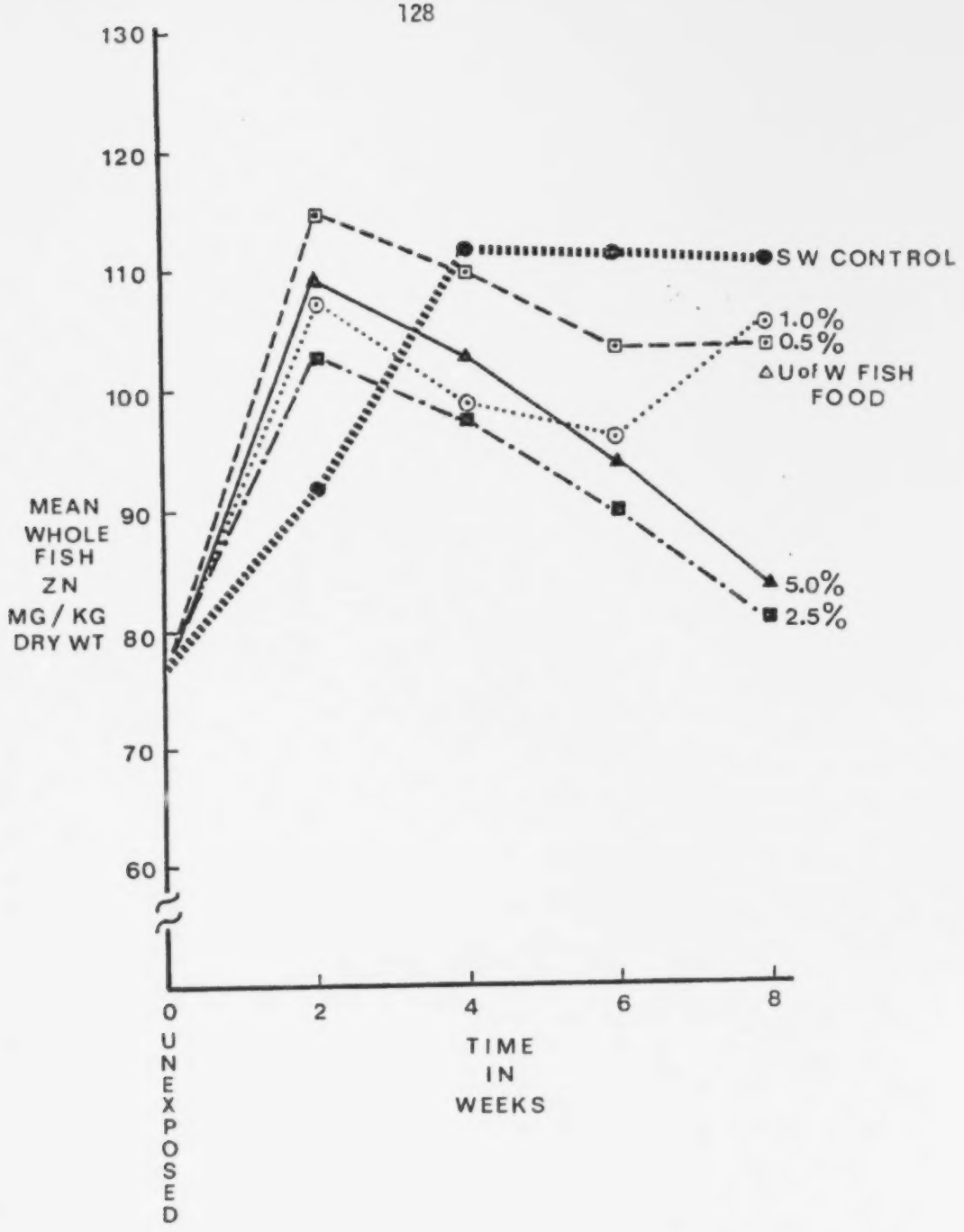


Figure 8. Summary of mean whole fish zinc content vs. time of exposure to sea water and 0.5, 1.0, 2.5, and 5.0% primary treated sewage effluent in sea water (v/v).

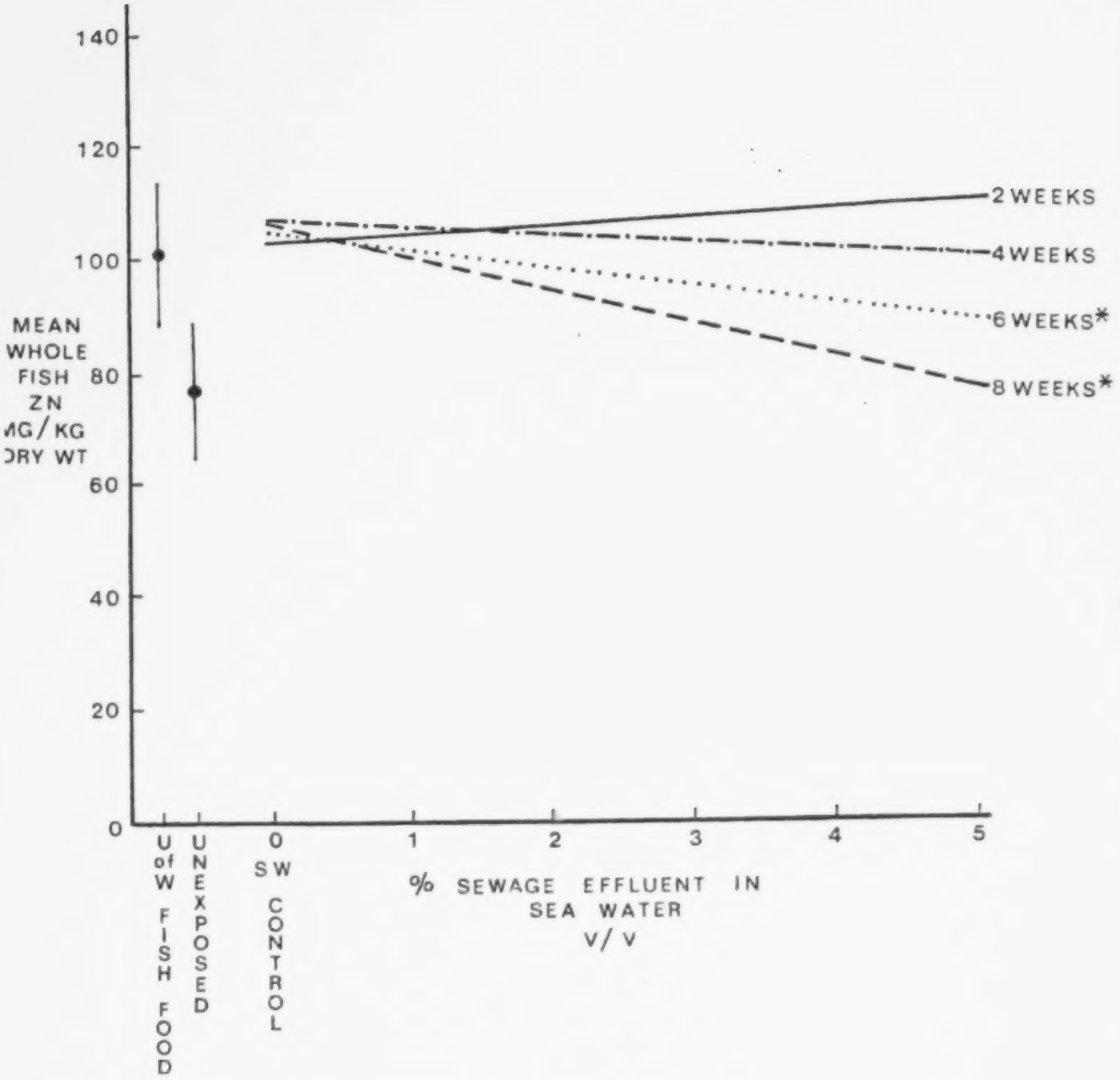


Figure 9. Summary of least squares lines of mean whole fish zinc content vs. exposure to dilutions of primary treated sewage effluent for 2, 4, 6, and 8 weeks. An asterisk (*) indicates the slope of the line was statistically significant at the .05 level of significance.

et al. (1973) found toxicity to golden shiners (Notemigonous chrysoleucas) to average 45% for primary sewage from four treatment plants in the San Francisco area, while Norris, et al. (1973) estimated the LC50 ranged from 20-33% for shiner perch in composite San Francisco wastewater effluent. Due to the wide variety of conditions under which other studies have been conducted, with primary focus of tests in freshwater, few valid comparisons can be made for marine species.

Treatment of the effluent by aeration or filtration did little to alter the toxicity. Tests of reduced salinity up to 60% v/v freshwater indicated that unusual stress was not occurring in the species tested due to reductions in the salinity.

The effect of increased temperature of the effluent on organisms in the receiving waters was probably minimal. Although the 96-h LC50 declined from 18 to 11% with a Δt of 10 C, a similar increase in toxicity was not expected in the mixing zone due to the initial 140:1 dilution.

The consistent sensitivity of shiner perch between age zero and 1^+ individuals made this species an attractive test animal for repetitive testing because it minimized one source of variance. However, tests with English sole indicated that the zero age group was at least twice as sensitive (96-h LC50 = 8%) when compared to the 1^+ age group. The age 1^+ English sole were equally sensitive as the shiner perch.

Ample data exist to show that chlorine is toxic to marine life in concentrations at the ppb level (Holland, et al., 1960; Stober and Hanson, 1974; Jolley, 1976; Stober, et al., 1976). Chlorine contributed a major component of the toxicity of the sewage which was demonstrated by dechlorination with SO_2. The 96-h LC50 for shiner perch and English sole approximately doubled. The toxicity of ammonia could not be tested independently because the treatment with clinoptilolite resin removed both ammonia and chlorine. Removal of both components substantially decreased the toxicity to both English sole and shiner perch.

Abnormally high mortality was observed during two bioassays, resulting in 96-h LC50 values of less than 10% v/v effluent. The exact toxic component(s) remain unknown, however, the mortalities did correlate with a peak in mercury on one occasion and a peak in chromium/copper on another. While copper and zinc occurred at consistent levels in the effluent, slug discharges of other trace metals occurred at infrequent intervals.

Chronic bioassays conducted for 8 weeks did not indicate a significant overall change in the shiner perch body burden of copper (Wert, 1977). The zinc body burden in shiner perch increased in both experimental and control fish probably due to the concentration in the food. Exposure to effluent less than or equal to 5% v/v concentrations did appear to alter the rate of accumulation and retention of whole body zinc. These data suggest that copper and zinc in solution may not be biologically available. However, the generally higher concentrations in fishes collected near the outfall (Olsen, 1976) indicated that the chemical state of the metals discharged may require alteration before bioaccumulation can occur, possibly through processing by decomposers followed by passage up through the food chain.

Blood chemistry and blood cell morphology of yearling coho salmon were adversely affected at 1.1 and 3.6% (Buckley, et al., 1976). The salmon were not affected in 0.3% effluent. Based on salmon blood chemistry and gill tissue pathology of shiner perch and English sole, 0.5% chlorinated effluent approximates an upper limit for the maximum acceptable concentration for discharge to Puget Sound receiving waters, if trace metals (i.e., mercury, chromium, copper) can be controlled.

ACKNOWLEDGMENTS

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EVALUATION OF MARINE INVERTEBRATE SPECIES DIVERSITY AS AN OIL TOXICITY INDICATOR FROM LABORATORY STUDIES

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ABSTRACT

Vanderhorst, J.R. and R. Wilkinson. 1978. Evaluation of marine invertebrate species diversity as an oil toxicity indicator from laboratory studies. Proc. Fourth Annual Aquatic Toxicity Workshop, Vancouver, B.C., November 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Artificial substrates colonized by marine algae and invertebrates were exposed for six months in the laboratory to continuously flowing sea water contaminated with No. 2 fuel oil. There were three sets of test conditions: (1) artificial light and oil; (2) artificial light and no oil; and, (3) natural light and no oil.

The results indicated significant depression ($p = 0.005$) of species diversity (H') and species richness (s) for oil-treated colonies as compared to controls. Naturally lighted communities were higher in species diversity than were those maintained under artificial lighting. Significant differences in relative abundance were not observed. A paired comparison of individual species occurrence patterns provided the most convincing measure of effects due to the fuel oil. Since major compositional shifts occurred in both treated and control colonies, a major shortcoming of species diversity or other single number indicators is clearly identified.

INTRODUCTION

The objective in this study has been to evaluate the relative usefulness of species diversity (Shannon-Weiner), relative abundance (Pielou), species richness, and individual species occurrence patterns for assessing effects on marine invertebrates in oil bioassay.

The bioassay of petroleum is especially challenging because oil and water do not form a homogeneous solution. Thus, expression of severity of treatment in oil bioassay based on volume to volume ratios has led to conflicting results depending on type of oil, method of application and behavior of test species (Vaughan 1973). Recent investigators have used measurements of waterbourne petroleum as an indicator of severity of treatment (e.g., Anderson et al. 1974; Cox and Anderson 1973; Rice and Short 1976; Vanderhorst et al. 1976; and others). Quantitative expression of severity of treatment in static or batch-treated bioassay is not practical for investigation of longer term exposures because of logarithmic decay of total oil concentration and undefinable shifts in contaminant composition in very short periods of time (1 to 6 hours, Vanderhorst et al. 1976). For these reasons, it is difficult to make meaningful comparisons in the relative vulnerability of different species of organisms to oil (Rice et al. 1976).

In the present studies, two approaches were combined to aid in making multi-species comparisons. First, to allow chemical characterization of the test solution, a continuous-flow apparatus (Vanderhorst et al. 1977a) was used with the objective of presenting stable concentrations and composition of contaminant. Second, by using colonies of marine algae and invertebrates as the test unit, simultaneous exposure of many species was accomplished to avoid differences in exposure conditions over a six-month period due to water quality, season, and contaminant availability.

MATERIALS AND METHODS

The exposure apparatus used (Fig. 1) has been previously described (Vanderhorst et al. 1977a). The No. 2 fuel oil was metered into a turbulent seawater stream for mixing (Chamber B, Fig. 1), and floatable oil was then discarded by surface riser pipes (Chambers B, C, D, Fig. 1). The nonfloating dispersion was metered to exposure tanks (F, Fig. 1) by adjustment of dripper arms attached to Chamber D (Fig. 1). Simultaneously, sea water was metered by a head tank and dripper arm manifold. Ratios of fuel oil dispersion (from Chambers B, C, D, Fig. 1) to sea water (from Chamber E, Fig. 1) were adjusted to obtain target concentrations of 0.1 and 0.6 mg/ℓ total oil as measured by IR spectrophotometry in exposure tanks (F, Fig. 1). Two 90 x 40 x 30 cm fiberglass exposure chambers received the higher concentration, two chambers received the lower concentration, and two received no No. 2 fuel oil dispersion. The exposure chambers each received a total of two ℓ/min combined No. 2 fuel oil dispersion and/or sea water. Continuous photoperiod was supplied

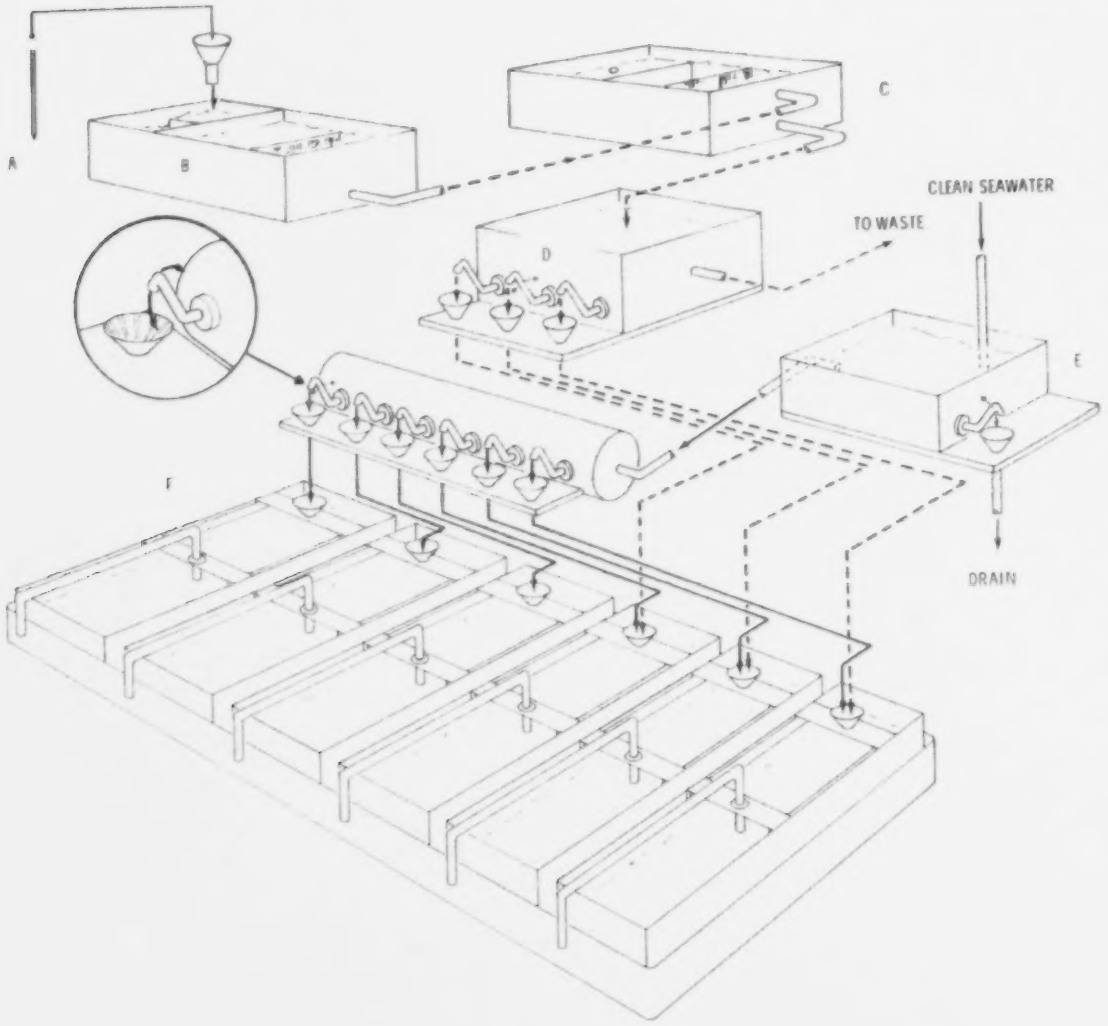

Figure 1. Exposure Apparatus Used for Bioassay of No. 2 Fuel Oil (After Vanderhorst, et al., 1977).

by two overhead 45-cm fluorescent tubes (Gro-lux^R) attached to each chamber. Three additional identical exposure chambers were maintained outside the laboratory, and differed from inside controls only in that they received ambient light. Detailed chemical characterization of the treatment media has been reported (Bean and Blaylock 1976; Vanderhorst et al. 1977b).

The units used for assessing biological effects under the above conditions were concrete construction bricks (19 x 9 x 6 cm) colonized by marine algae and invertebrates. A large pool of bricks (500) were allowed to colonize at mean lower low water, Sequim Bay, Washington, over winter. In April, 121 bricks were selected for use in the experiment based on the criterion that they have a full cover of the green alga, *Ulva sp.* Strict random procedures were used to select colonies from this reduced pool for the different exposure conditions. Six bricks were assigned to each test chamber and arranged in rows of two. Monthly (July through December), a brick from a randomly chosen position, common to all test chambers, was removed. Organisms were identified, counted and weighed. Six additional colonies were evaluated prior to commencing the experiment.

Since all treated and control chambers received a continuous supply of raw (unfiltered) sea water, the system was open to recruitment throughout the course of the experiment. The seawater supply came from 3 meters below MLLW, Sequim Bay, Washington, adjacent to the beach used for initial colonization. Salinity, pH, and dissolved oxygen varied little during the experiment. Typical values were 30 ppt (salinity), 8.0 (pH), and 8.6 mg/ℓ (dissolved oxygen). Temperature exhibited a seasonal trend from 11.5 degrees C in June, to 13.2 in August, and a low of 8.3 in December.

RESULTS

Data from measurements of oil concentration and composition in this experiment revealed significantly different concentrations in total oil between the two target concentrations (0.1 and 0.6 mg/ℓ) and that the average magnitude was correctly predicted (Bean and Blaylock 1976; Vanderhorst et al. 1977b). Composition of the No. 2 fuel oil was similar in measured parameters to parent fuel oil since there was not an enhancement of the toxic aromatic components. A preliminary analysis of species diversity and species richness indicated significant differences in these parameters between control tanks and treated tanks but did not indicate differences between tanks receiving the two target concentrations (Vanderhorst et al. 1977b).

Since high variability in response parameters was encountered in the earlier work (Vanderhorst et al. 1977), we wished to combine as replicates the four tanks at the two treatment concentrations and four of the five controls for making comparisons. We selected the four controls for use in this study, as well as four of the six pretest colonies, by strict random procedures. Figure 2 shows linear regressions of species diversity ($H' = \Sigma P_i \log_2 P_i$) on month of experiment calculated for the individual and combined control and treatment conditions. Distinct trends for control versus treatment tanks are

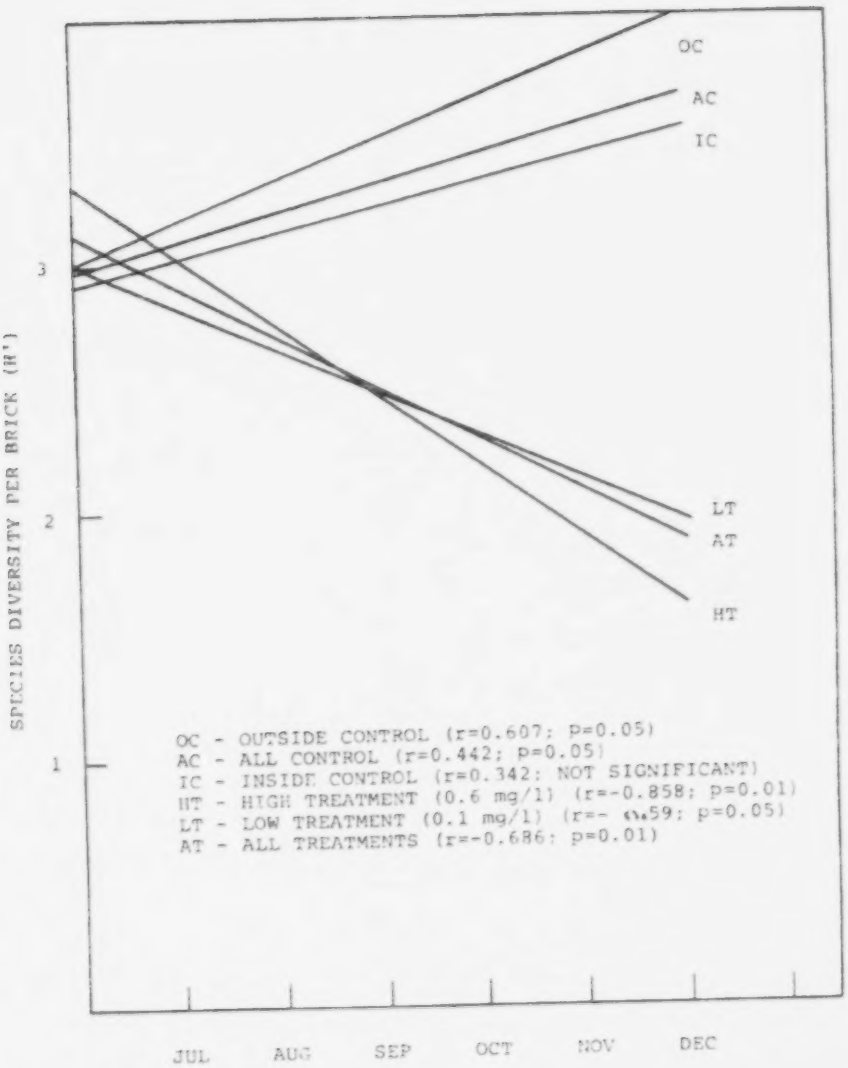

Figure 2. Relationship of Species Diversity (H') to Month of Exposure to No. 2 Fuel Oil.

apparent. All control regressions have a positive slope of diversity on time. For ambient lighted controls, and for combined controls, the slope significantly ($p = 0.05$) differs from zero. For inside (artificially lighted) controls, the calculated positive slope is not significantly different from zero. The values for $\bar{r}$ give some idea of the amount of scatter observed in data. All treated conditions (individual concentrations and combined concentrations) exhibit significant ($p = 0.05$; 0.01) negative slopes of diversity in time. From these data we felt justified in using the various control and treatment conditions as replicates for further analysis, and we conclude that treatment with No. 2 fuel oil significantly depressed species diversity (H').

We chose the Shannon-Weiner formula for species diversity because it is, perhaps, the most popular index in use for pollution assessment (Vanderhorst and Wilkinson 1974). Investigators recognize two components in diversity as expressed by H', i.e., species richness (some measure of the kinds of organisms) and evenness or equitability (the distribution of an importance factor for individual species among all species). In this study, we used numbers of individuals as an importance factor to compute the Pielou index of evenness or relative abundance (H'/H'Max). Figure 3 shows the linear regressions of relative abundance over time for the combined data of treatments ($n = 26$ pairs) and controls ($n = 26$ pairs). We observed a positive slope in relative abundance for controls which is significantly different from zero ($p = 0.05$). Treated tanks did not exhibit a significant trend in relative abundance. The slight, but significant, increase in relative abundance for control tanks indicates that distribution of individuals among the species became more nearly even as the experiment progressed. Since the average numbers of individuals per species on pretest bricks was quite small (Table 1), and a single species, *Alvinia sp.* contributed nearly half the individuals, we anticipated that removal of this species would dramatically influence the index value. A regression over time of the numbers of *Alvinia sp.* in control tanks is presented on Figure 4. The loss of numbers for this species through month of experiment is highly significant ($p = 0.01$). Seemingly, this might account for the significant increase in relative abundance as a function of time. To the contrary, on Figure 5, we plotted the linear models of relative abundance with *Alvinia sp.* included (WA) and without *Alvinia sp.* included (WOA). Even though this plot is shown on an expanded scale, the sensitivity of the index to this major numerical contributor is slight.

We turn now to the second component of species diversity, i.e., species richness. Although several indices are available for measurement of species richness, e.g., Margalef (1958); Menhinick (1964); Odum et al. (1960), a common practice, and the one followed here, is to express richness not as an index, but simply as the number of species per brick. First, on Figure 6, is the relationship of numbers of species to time in the combined control groups. We have classified as "original" species those species which occurred in the pretest sample (Table 1) and "new" species as those which appeared later. From Figure 6, the total number of species was not significantly different in controls as a function of time. Likewise, the number of original species did not significantly ($p = 0.05$) change during the experiment;

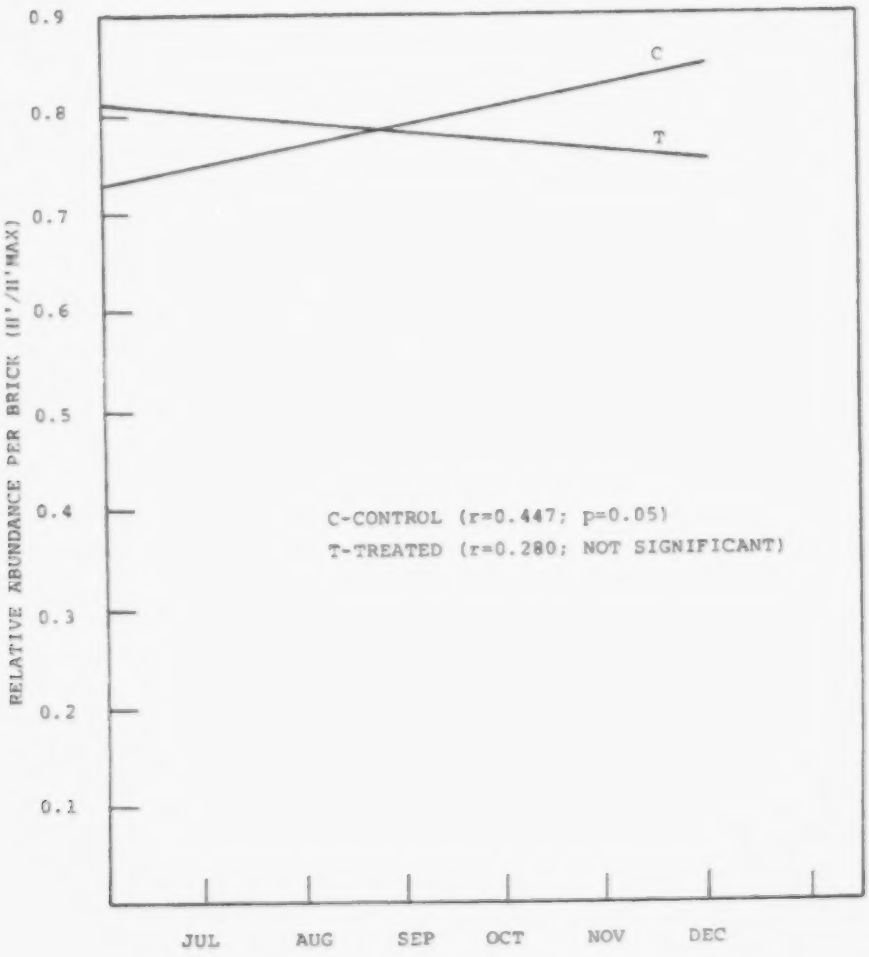

Figure 3. Relationship between Relative Abundance Index (H'/H'Max) and Month of Exposure to No. 2 Fuel Oil.

TABLE 1. Numbers of individuals per brick representing species on pretest colonies used for No. 2 fuel oil experiment

SPECIES/GROUP	AVERAGE NUMBERS/BRICK
Platynereis bicanaliculata	0.33
Lepidonotus caelorus	0.50
Prionospio sp.	0.33
Armandia bioculata	0.16
Lumbrinereis sp.	0.16
Leptocheila savignyi	1.00
Emplectonema gracile	0.16
Exosphaeroma amplicauda	1.83
Aoroides columbiae	1.16
Parallorchestes ochotensis	16.33
Ampithoe simulans	1.00
Ampithoe sp.	0.16
Amphipods (undet.)	9.50
Pagurus hirsutiusculus	0.16
Pagurus sp.	0.16
Pinnixia faba	0.16
Pinnixia occidentalis	0.33
Pugettia gracilis	0.16
Pagurietes sp.	0.16
Mytilus edulis	0.16
**Alvinia sp.*	43.66
Lacuna sp.	9.33
Odostomia sp.	0.33
Bittium sp.	0.33
Nassarius sp.	0.16
Margarites sp.	2.16
Acmaea pelta	0.16
Acmaea persona	0.50
Acmaea digitalis	0.50
Acmaea scutum	0.33
Cooperella subdiaphana	0.50
Mysella tumida	4.17
Hiatella arctica	0.16
Mopalia lignosa	0.33
Lepidozona sp.	0.16
Cyanoplax hartwegii	0.16
Katharina tunicata	0.16

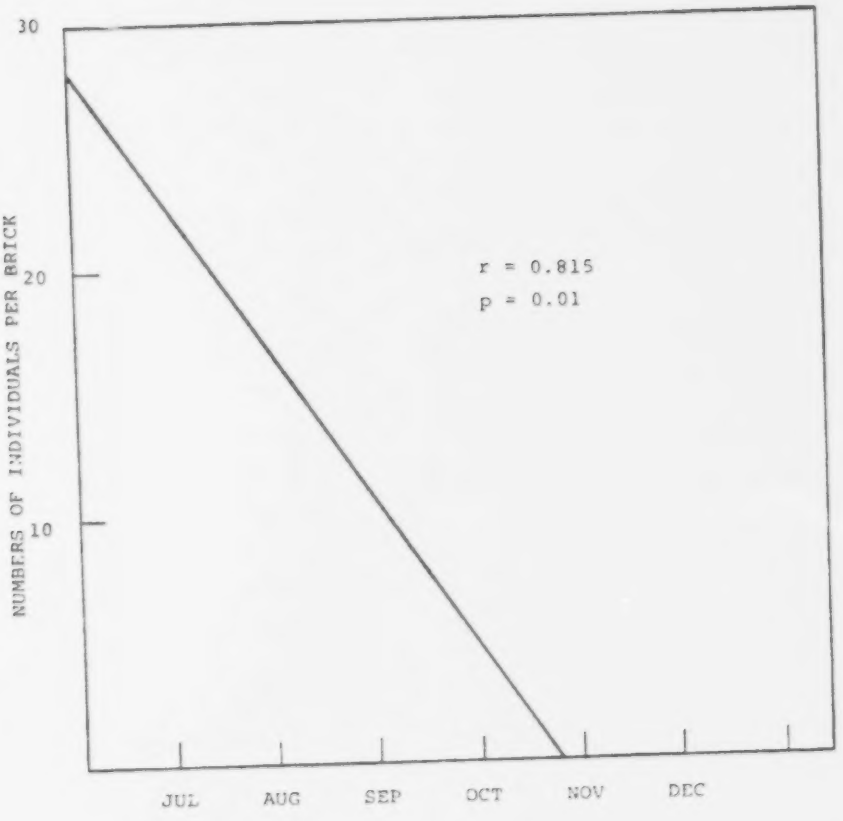

Figure 4. Relationship of Numbers of *Alvinia* sp. to Month of Exposure to No. 2 Fuel Oil.

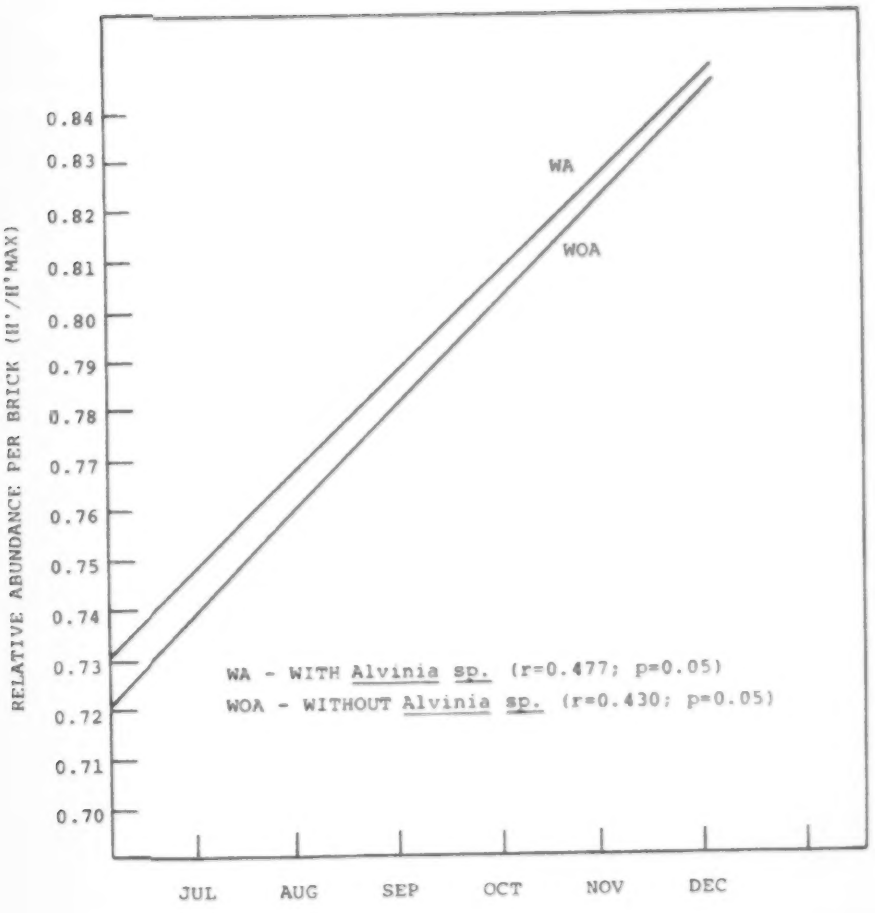

Figure 5. Comparison of relative abundance (H'/H' Max) with *Alvinia* sp. included and not included.

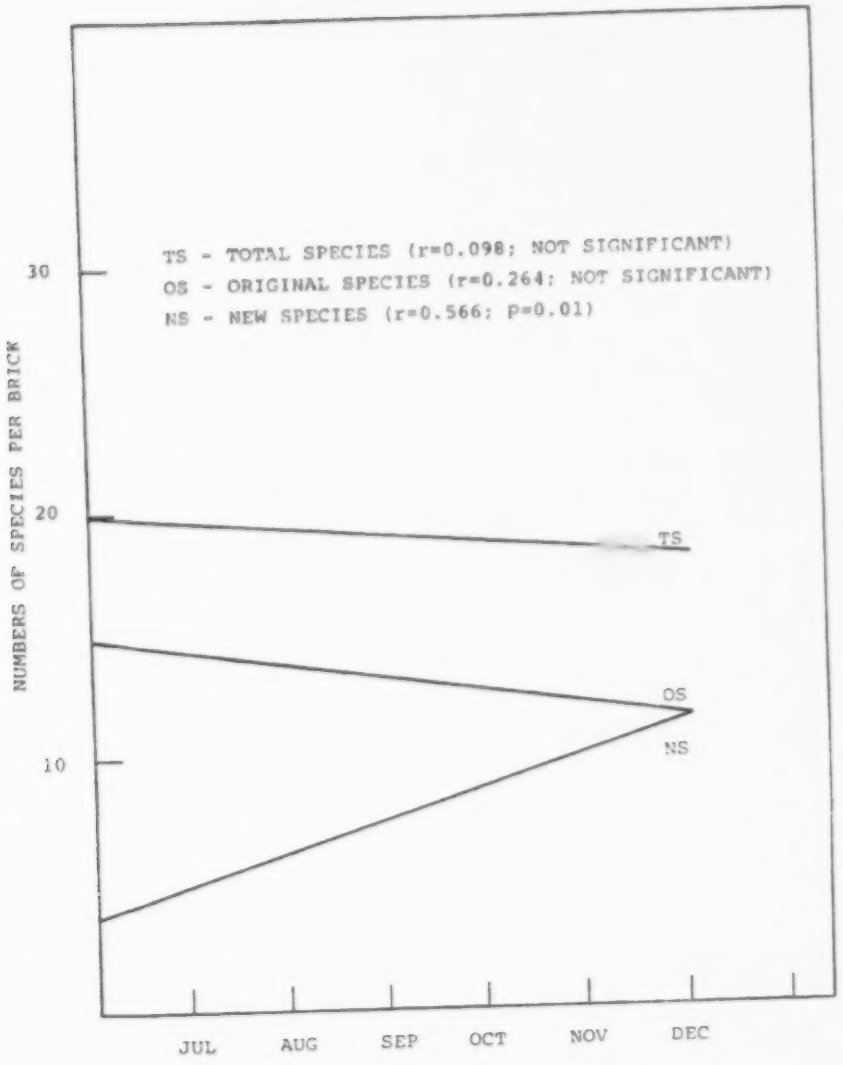

Figure 6. Relationship of Numbers of Species/Brick to Month of Experiment in Control Tanks.

however, the computed slope is negative. New species had a highly significant ($p = 0.01$) increase in contribution as the experiment progressed. At termination of the experiment, new species amounted to 63% of the total. Data on species richness for treated colonies is plotted on Figure 7. From these plots, it is apparent that total numbers of species and original species exhibit significant ($p = 0.01$) declines on treated colonies. The numbers of new species did not significantly increase as a function of month as was the case for controls (Figure 6).

The data on numbers of species (Figs. 6 and 7) direct attention to the problem of measuring which species were on the controls and not on treated colonies. To this end, we created contingency tables based on the frequency of occurrence of individual species on control colonies on a monthly basis. For this purpose, we used only the inside (artificially lighted) control tanks. Measurement of the probability of observed differences in monthly occurrence being real were computed using the Chi square statistic. Table 2 tabulates those species for which the probability of a real difference in occurrence pattern exceeds 75%. With the exception of the polychaete, *Cirratulus cirratus*, all species so classified occurred more frequently on controls. We have also indicated on Table 2 whether or not the included species were present in the pretest sample (Table 1).

DISCUSSION

Data have been presented on the response of intertidal colonies to a continuous low concentration of No. 2 fuel oil in terms of: (1) species diversity; (2) relative abundance; (3) species richness; and (4) frequency of individual species occurrence. It has been the purpose of this study to allow comparisons in the utility of the responses observed for evaluating the effects of No. 2 fuel oil under conditions in which controlling factors other than the oil have been given equal probability for influence. The laboratory system was open to recruitment, a feature which more nearly parallels natural systems than would a closed laboratory system. We do not purport that the system is an accurate reflection of colonizing conditions in the marine intertidal zone because no provision was made for duplicating tidal cycles and the seed water was drawn from a slightly greater depth than the tidal height of colonization. Since extraneous factors other than No. 2 fuel oil were randomized across the experimental design, comparisons in sensitivity to effects of the oil are valid.

We observed a significant depression in species diversity (H') in No. 2 fuel oil treated colonies compared to controls. A considerable literature has developed describing effects from pollution in terms of this and other species diversity measures. Pertinent to the here-reported work are the proposals (with a large following) of some authors, e.g., Wilhm and Dorris (1968); Cairns and Dickson (1971) to consider a specific index value as indicative of "polluted" or "healthy" conditions. The data from Figures 2 and 6 clearly indicate a major shift in species composition in face of a

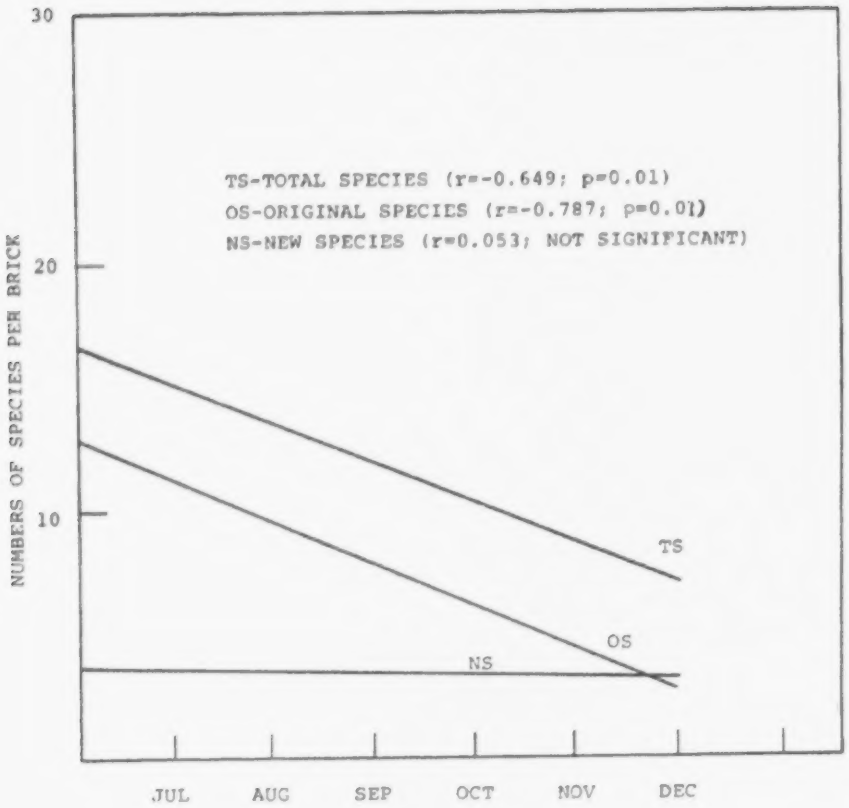

Figure 7. Relationship of Numbers of Species/Brick (Combined Treatments) to Month of Exposure to No. 2 Fuel Oil.

Table 2. Groups with differences in occurrence patterns between No. 2 fuel oil treated and control colonies.[1]

GROUPS/SPECIES	PRESENT ON PRETEST COLONIES	PROBABILITY OF DIFFERENCE: TREATED vs. CONTROL (%)
SEGMENTED WORMS	Yes	75
Thelepus sp.	No	95
Boccardia sp.	No	95
Cirratulus cirratus[2]	No	75
CLAMS	Yes	95
Cooperella subdiaphana	Yes	99
Clinocardium nuttalii	No	75
Hiatella arctica	Yes	75
TANADACEANS	Yes	95
Leptocheila savignyi	Yes	95
AMPHIPODS	Yes	99
Acroides columbiae	Yes	99
DECAPODS	Yes	99

[1] Based on contingency tables and Chi-Square statistic for paired data.

[2] Occurred more frequently in treated colonies; all others more frequent on controls.

rising index of diversity in controls. From the species richness data (Figure 6) one can state with some certainty that the compositional shift occurred; one cannot infer that the shift reflects "healthy" or "unhealthy" conditions. We suggest that the diversity index, which fails even to detect the compositional shift, would be of less reliability as an indicator of community health. Rather, the data indicate that the index, per se, was increased on controls and depressed by No. 2 fuel oil treatment.

Two sets of dichotomies arise when one wishes to interpret the meaning of gross differences between control and experimental species diversity (H'). First, there is a basic ambiguity in the index sinc a depressed comparative value brings to mind not one, but two possibilities, i.e., the proportions of individuals within all species may have shifted toward inequality in treatments (thus a declining relative abundance factor); or, the numbers of species may have decreased in treated colonies. The second dichotomy arises because in the present study, the species diversity index (H') in controls revealed a significant increase through the course of the experiment. Thus, gross differences in values between treatment colonies and experimental colonies may reflect on one hand the increase in control values or, on the other, the significant decrease in treatment values.

Because of the dichotomies stated above, intuitive interpretation of the meaning of gross differences in control and treatment diversity (H') seems impossible. An examination of the proportional contribution of species (H'/H'Max, Fig. 3) indicates a significant increase in controls. This increase parallels the increase in the parent index (H'). There was not a significant change for H'/H'Max in treated colonies. The control data suggests that one or a few numerically dominant species had diminishing contribution over the course of the experiment or that species with few numbers gained in abundance. A cursory examination of Table 1 reveals that the numbers of individuals per species per brick was initially very low, and that the snail, *Alvinia sp.* contributed about one half of all individuals on pretest colonies. A linear regression of numbers of *Alvinia sp.* on month of experiment is negative and highly significant (Fig. 4). Thus, it appeared that this would be a possible explanation for the rising index value (Fig. 3). In fact, a second computation of relative abundance with the numerically dominant snail removed (Fig. 5) did not change the form of the relationship or the significance of the linear model, and only slightly changed the magnitude. For this reason, we conclude that the rise in control diversity (H') and relative abundance (H'/H'Max) are not explained by the present analysis; the relative abundance component was not particularly sensitive to the complete removal of a numerically dominant form; and, that index is not a good indicator of the effects of No. 2 fuel oil. Since this general conclusion is in contrast to the role of the evenness component (Pielou, 1975), our data (Fig. 5) merely provide a measure of the relative degree of sensitivity one may expect from the index value in response to the loss of a numerically dominant species.

Species richness, the other component of diversity (H') does provide information which is amenable to interpretation. We have alluded above to the dramatic compositonal shift in control colonies, and have pointed out that

the total numbers of species in control did not significantly change during the experiment. From this we conclude that the species richness component did not contribute significantly to the rising control value of H'. An examination of the total numbers of species on treated colonies reveals a significant decline which parallels the H' values on treated colonies (Fig. 2). Thus, the primary action of No. 2 fuel oil on H' was to reduce the species richness component. It should be pointed out that in order to compute H', it is first necessary to compute the data on numbers of species. Thus, the finding concerning effect on H' is ancillary to the primary data.

The difference in linear regressions for new species in controls (Fig. 6) and on treated colonies (Fig. 7) provide an estimate of the magnitude of the effect on recuitment to the system by No. 2 fuel oil. Since new species in controls amounted to 63 percent of total species by the end of the experiment and no significant increase in new species occurred for treated colonies, recruitment to the system is identified as a major factor. It obviously makes it impossible to adequately define duration of exposure of species to the No. 2 fuel oil. It suggests that either an alteration of substrate occurred which precluded successful settlement and/or mortality of entering larval forms was high. These are important areas for future investigation in both laboratory and field studies. The data also suggest that the most useful measure of effects from this study with No. 2 fuel oil lies in month-paired comparisons.

The paired comparisons of species occurrence (Table 1) provide a built-in importance factor for species which are determined to have been influenced by treatment. This is so since a species must have a very frequent occurrence in controls to have high probability of being influenced by treatment, i.e., they are dominant in occurrence. Species which have apparently high sensitivity to No. 2 fuel oil but do not occur with high frequency are not detected by the statistical test. Thus, for 22 species of amphipods which occurred in controls, none of which occurred in treated colonies, only differences in occurrence for the species, *Aoroides columbiae*, were significant. Thus, the approach has identified species with a high vulnerability in terms of availability and sensitivity to the effects of No. 2 fuel oil. We believe that the data on paired occurrence represents the most useful of data obtained in this study.

ACKNOWLEDGEMENT

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Lethal and Sublethal Toxicities of Copper-Nickel Mixtures to the Zebrafish *Brachydanio rerio*

by

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ABSTRACT

Weinstein, N.L., and P.D. Anderson, 1978. Lethal and Sublethal Toxicities of Copper-Nickel Mistures to the Zebrafish *Brachydanio rerio*. Proc. Fourth Annual Aquatic Toxicity Workshop. Vancouver, B.C., Nov. 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

An approach to assessing quantitatively the toxicities of mixtures of poisons is employed for studying the multiple toxicities of copper and nickel to the zebrafish (*Brachydanio rerio*). Bioassays were performed at lethal and sublethal concentrations. The response variable of the lethal bioassays was percentage mortality, a quantal factor, while the sublethal response parameter was graded in terms of fecundity.

Lethal and sublethal mixtures of copper and nickel evoked supra-additive effects, which proved to be substantially greater in magnitude than the effects predicted according to a hypothesis of toxicant additivity.

Agencies which prescribe permissible levels of chemical pollutants are cautioned as to the potential inadequacies of current standards in terms of protecting aquatic organisms against unique and/or highly potent forms of toxicity which may characterize certain mixtures.

INTRODUCTION

Commonly, organisms inhabiting the receiving waters of man-made or man-mobilized wastes are exposed to a variety of chemical pollutants. This multiple-contaminant exposure frequently results in the expression of unique and/or highly potent forms of toxicity which would not occur if the mixture constituents were operating discretely. It follows therefore, that water quality standards which set permissible levels based on the potencies of discrete toxicants often do not protect aquatic organisms exposed either sequentially or concurrently to mixtures of chemical pollutants.

This study addresses the problem of assessing quantitatively the lethal and sublethal toxicities of mixtures of copper and nickel. The concurrence of man-mobilized copper and nickel has been documented for such watersheds as the Sudbury mining region (Stokes, *et al.*, 1973; as cited by Hutchinson *et al.*, 1976; Stokes, 1975) where the potentially hazardous prevalent levels of these heavy metals are a cause for concern.

METHODOLOGY

The overall format of the study consisted of bioassays exposing groups of test organisms to copper and nickel in discrete solutions and in mixtures. These tests were conducted at lethal and sublethal concentrations. The response variable of the lethal experiments was percentage mortality, a quantal factor, while the sublethal response parameter was quantified in terms of numbers of eggs produced, a graded variable.

a) Quantal Responses

Our approach to the study of lethal multiple toxicity is based on the methodology applied by Anderson and Weber (1975). Quantal response curves, generated by plotting probit mortality against the common logarithm of concentration (Finney, 1971), are derived for each of the consitituents of the heavy metal mixture and compared for parallelism. Parallel curves infer that the variances of the respective tolerance distributions for the individual poisons are identical, a condition which is presumably manifested by similarly acting toxicants (Bliss, 1939; Finney, 1971). Similarly acting poisons are thought to operate by evoking identical

affinities and intrinsic activities on the same target receptors (Fig. 1). The possible effects due to interactions between two similarly acting toxicants are illustrated in Fig. 2.

Responses to lethal mixtures of copper and nickel are expressed as probit mortality and plotted against the common logarithm of mixture concentrations. The latter are derived by the following formula in accordance with a hypothesis of strict addition.

$$\text{Log } C_A = \log (Ni^{++} + R\ Cu^{++}) \quad (1)$$

where C_A = "additive" concentration for mixture

Ni^{++} = concentration of nickel in mixture

Cu^{++} = concentration of copper in mixture

R = relative potency between nickel and copper, computed as the abcissal difference between discrete response curves for copper and nickel

Equation (1) is based on the assumption that copper and nickel contribute to the overall potency of a mixture in proportion to their relative potencies. The term $R\ Cu^{++}$ mathematically converts copper concentrations in mixtures to equipotent nickel concentrations. The sum of the converted copper value added to the actual nickel concentration in a mixture is the theoretical additive concentration, expressed in terms of an equipotent nickel concentration. Thus, response levels predicted according to the hypothesis of strict addition are computed using the equation derived from the quantal bioassay exposing *B. rerio* to discrete solutions of nickel.

b) Graded Responses

Responses to sublethal levels of copper and nickel in discrete solutions are graded in terms of percentage reduction in numbers of eggs (as compared to control numbers of eggs) and plotted against the common logarithm of toxicant concentration in a linear regression model. The authors deem this to be a reasonable methodology in that many graded biological responses demonstrate a linear trend when correlated with the common logarithm metameter of stimulus. Muska (1977), investigating the sublethal effects of heavy metals on the growth of guppies, applied a similar model to quantify his results, which he expressed as a linear function of the natural logarithm of concentration.

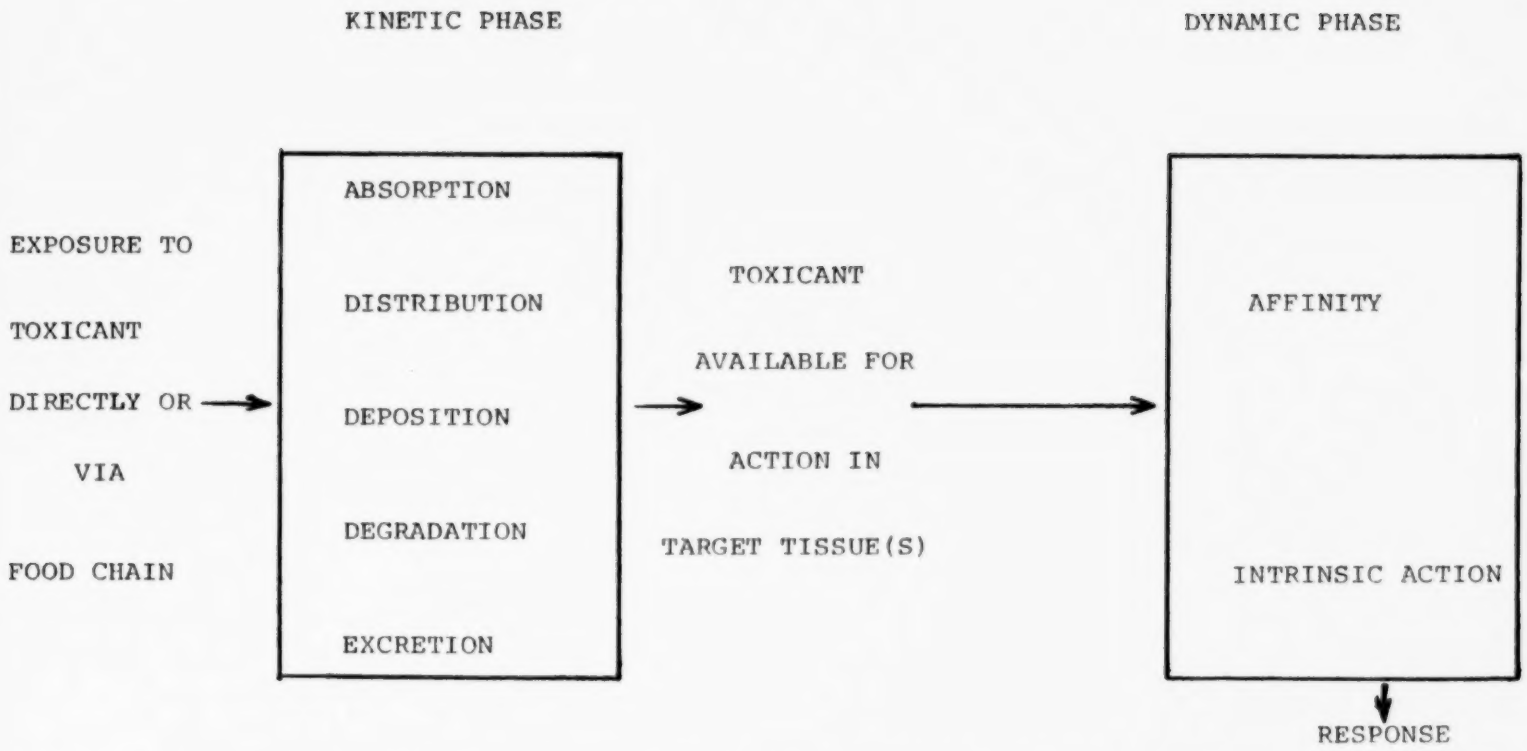

Fig. 1. Schematic illustration of the fate (kinetic phase) and toxic action (dynamic phase) of a chemical contaminant assimilated by an organism directly from the ambient environment or from the food chain (Modified from Ariens, 1972).

POSSIBLE KINDS OF INTERACTIONS BETWEEN TWO SIMILARLY ACTING TOXICANTS, A and B

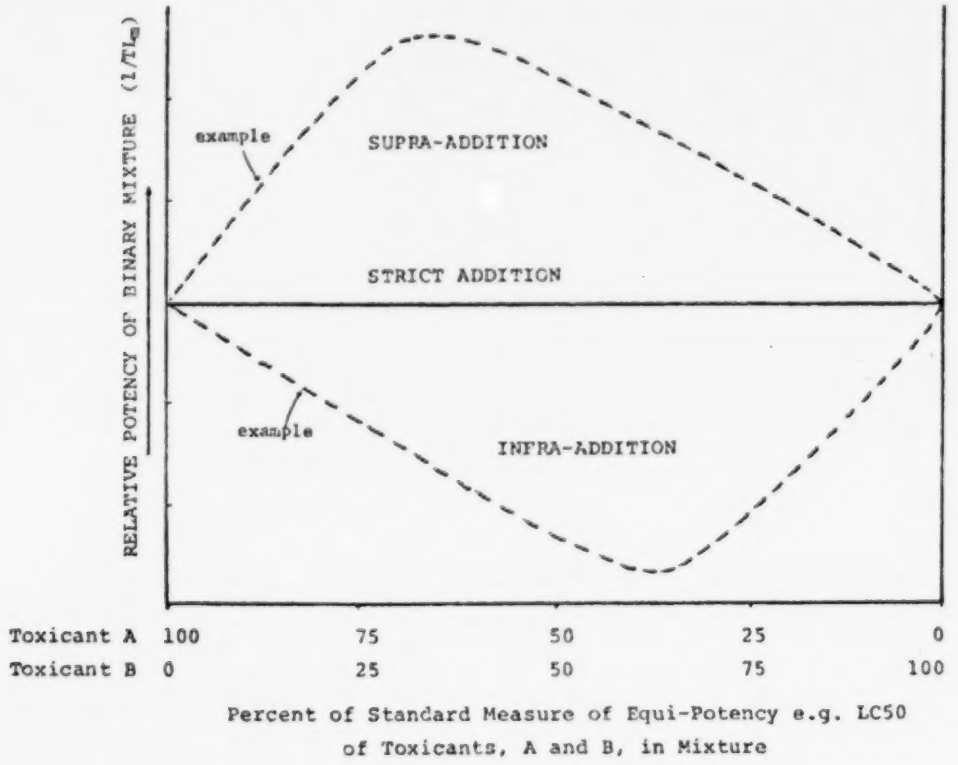
RELATIVE POTENCY OF BINARY MIXTURE ($1/TI_m$)
example
SUPRA-ADDITION
STRICT ADDITION
example
INFRA-ADDITION
Toxicant A 100 75 50 25 0
Toxicant B 0 25 50 75 100
Percent of Standard Measure of Equi-Potency e.g. LC50
of Toxicants, A and B, in Mixture

Fig. 2. Isobolograms representing three possible forms of multiple toxicity for binary mixtures of similarly acting toxicants. (Modified from Warren, 1971).

Responses to hypothetically sublethal mixtures of copper and nickel are plotted against the common logarithm of additive concentrations which are computed by equation (1) and expressed as equipotent nickel concentrations. Responses predicted according to the hypothesis of strict addition are therefore calculated by the equation derived from the sublethal bioassay using discrete solutions of nickel.

PROCEDURES

The zebrafish *Brachydanio rerio* (Hamilton-Buchanon) was chosen as a test organism for several reasons: (1) it is small, about 4.5 cm. at maturity, and easy to handle in the laboratory; (2) it is a continuous breeder that produces large numbers of emersible, non-adherent, and transparent eggs; (3) the timing of egg laying may be regulated by photoperiod.

Prior to all bioassays the test organisms were acclimated to control conditions for at least two weeks. Photoperiod was maintained as a 12-hour light - 12-hour dark cycle.

Toxicity modifying factors such as water temperature, pH, dissolved oxygen, hardness, and alkalinity were measured periodically and were found to remain fairly constant throughout each experiment. Table 1 lists some water quality characteristics.

In preparation for the lethal bioassays test organisms were starved for 24 hours prior to onset of the tests and for the duration of the 96-hour exposure periods. Batches of 10 or 14 fish were assigned to each 50-litre glass test tank.

For the sublethal bioassays total numbers of eggs produced by lots of five females and five males were enumerated daily and compared for a 10-day control period and a subsequent 10-day exposure period. Thus, each group was observed as its own control. Batches of breeding fish were confined to breeding traps in order to isolate the eggs laid from the egg-eating adults. The feeding regime consisted of a first daily feeding of Tetramin Staple tropical fish food, followed by a second daily feeding, several hours later, of a blend of dried ground beef liver, calf heart, and trout chow.

Table 1. Water Quality Data

Analysis of Laboratory Water Used in Experiments

dissolved oxygen	% sat.	87
temperature	^{o}C	25
pH		7.7
alkalinity	mg/l $CaCO_3$	105
hardness	mg/l $CaCO_3$	105

Analysis Performed at the City of Montreal Filtration Plant

colour	STD	5
turbidity	JTU (formazin)	0.4
total residue	103°C, mg/l	190
loss on ignition	550°C, mg/l	92
silica	mg/l SiO_2	37.4
calcium	mg/l Ca	37.4
magnesium	mg/l Mg	8.1
sulfates	mg/l $SO_4=$	26
chlorides	mg/l Cl^-	27
sodium	mg/l Na	12.3
potassium	mg/l K	1.4
fluorides	mg/l F^-	0.15
iron	mg/l Fe	0.012
carbon dioxide	mg/l CO_2	0.3
detergents	LAS	0.017

Stock solutions of cupric and nickelous sulfates were dripped from separate Mariotte bottles into a plexiglass diluting apparatus (Anderson and Weber, 1975), which was designed to dilute each stock solution discretely and independently before combining them in mixtures. The apparatus was adjusted to deliver the diluted solutions separately or in mixtures at a rate of 300 ml/min. to each 50-litre glass aquarium in a continuous flow-through system. This exchange rate ensured 90 percent replacement of water in less than 6 hours (Sprague, 1973).

Daily samples of the toxicant test solutions were taken from exposure tanks and analyzed subsequently on a Perkin Elmer #503 atomic absorption spectrophotometer. Flame and flameless (graphite furance) techniques were employed to measure high and low concentrations respectively. Mean concentrations of the heavy metals were computed for each lot of test organisms and used in subsequent calculations.

RESULTS

a) Quantal Bioassays

Quantal response curves relating probit mortality to log concentrations of copper or nickel do not deviate significantly from parallelism (p=.05), suggesting that these toxicants may have been similarly acting at lethal levels to *B. rerio*. Quantal curves adjusted to a common slope (Finney, 1971) are represented in Fig. 3.

Fig. 4 reveals the results of bioassays with lethal mixtures of copper and nickel. This graph illustrates the observations that actual responses in terms of probit mortality far-exceeded the responses predicted according to the hypothesis of strict addition.

b) Graded Bioassays

The mean number of eggs enumerated over the 10-day control period per group of five females and five males was 3000. Linear regressions relating percentage reduction in total numbers of eggs to log concentration of copper or nickel do not deviate significantly from parallelism (p=.05). Sublethal response curves adjusted to a common slope are depicted in Fig. 5.

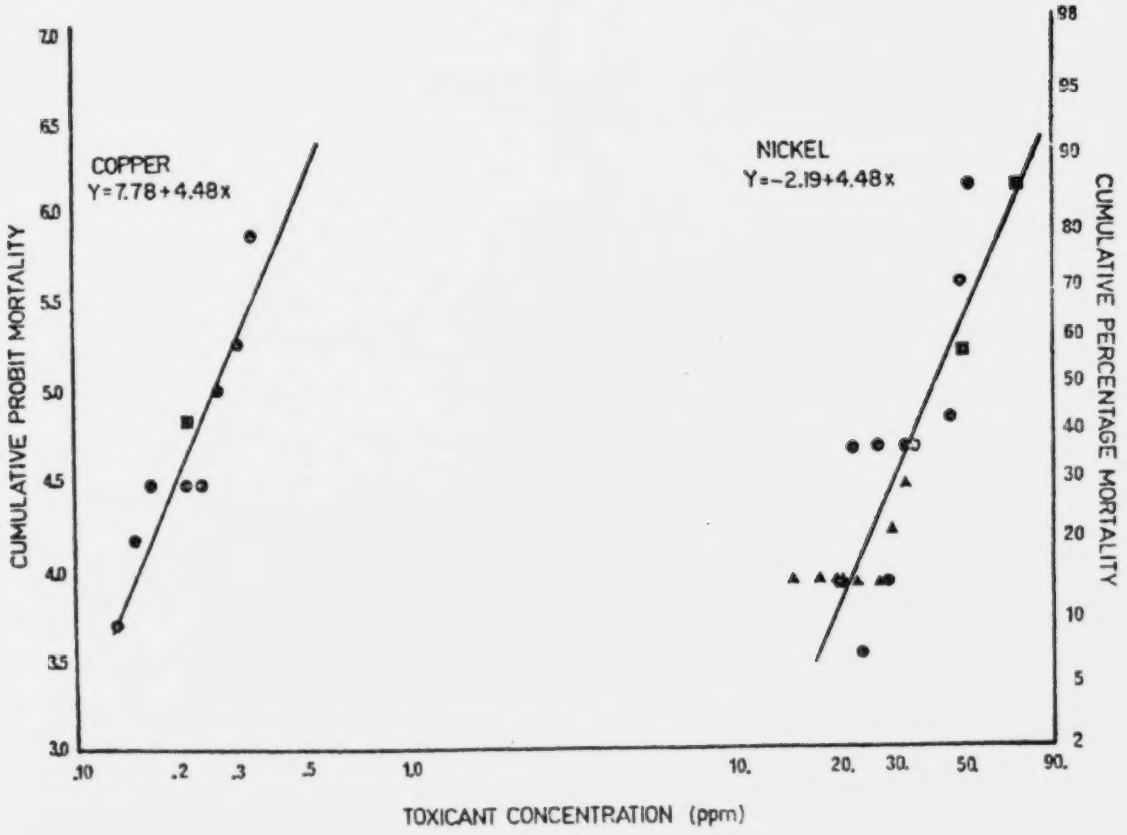

Fig. 3. 96-hour lethal response curves for *B. rerio* exposed in a continuous flow-through system to copper or nickel in discrete solutions. Different symbols represent different experiments. Regression lines are fitted by probit analyses and constrained to parallelism (Finney, 1971).

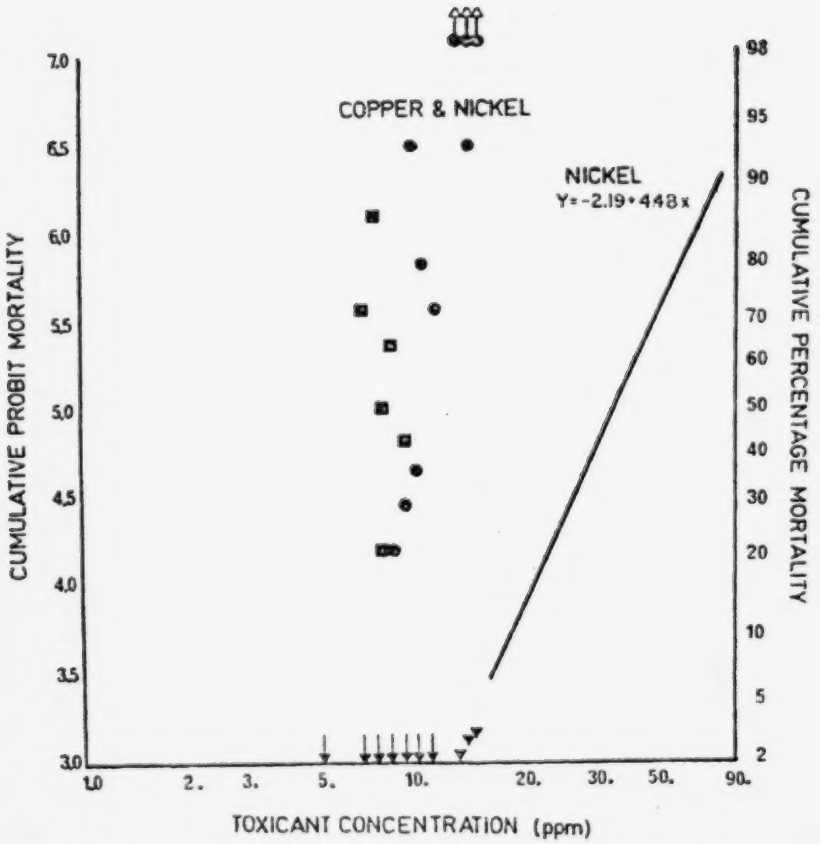

Fig. 4. 96-hour lethal responses of *B. rerio* exposed in a continuous flow-through system to mixtures of copper and nickel. Observed responses to mixtures are plotted against C_A, the additive concentration computed by Equation (1). Different symbols represent different experiments. Open arrows at top of graph designate observations of 100% mortality. Predicted responses, derived in accordance with the hypothesis of strict addition, are depicted on the graph by darkened arrows, many of which fall below 2%.

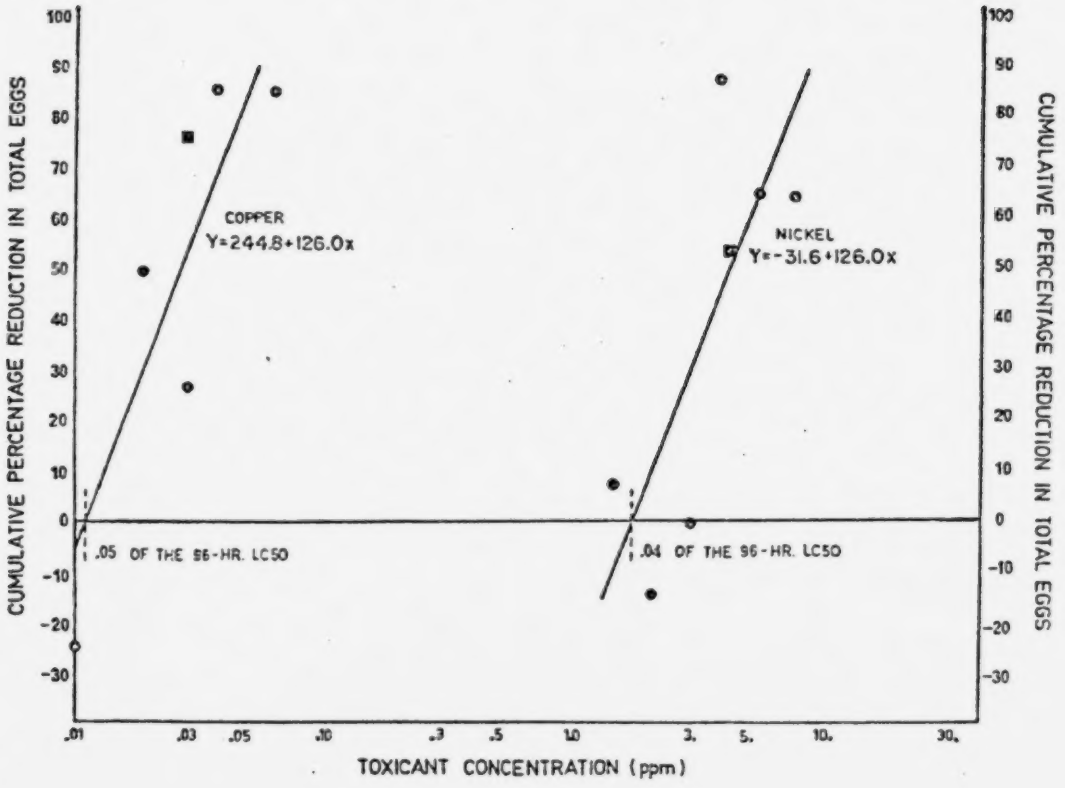

Fig. 5. 10-day sublethal response curves for *B. rerio* exposed in a continuous flow-through system to copper or nickel in discrete solutions. Points representing percentage reduction in total numbers of eggs (per breeding culture of 5 males and 5 females) are fitted by linear regressions constrained to parallelism.

The results of bioassays testing hypothetically sublethal mixtures of copper and nickel are represented in figure 6. Observed responses in terms of percentage reduction in total numbers of eggs were substantially greater than response levels predicted by the strict addition hypothesis. Fig. 6 also depicts the occurrences of mortality (ranging from 10% to 90% of the individual groups) which were observed within 96 hours of exposure to four of the higher hypothetically sublethal mixture concentrations.

DISCUSSION

The degree of supra-additivity is not expressed numerically for all points on the graphs (Figs. 4 and 6) depicting the results of the multiple toxicity bioassays because these points represent a series of toxic mixtures in which the relative potency ratio between the copper and nickel constituents was not constant. This precludes the fitting of a linear regression line which is only justifiable if the relative potency ratio between the constituents of the mixture is identical for all mixtures. A mathematical model which accounts for discrepancies in potency ratios and effectively formulates dose-response curves for infra or supra-additive binary mixtures has been derived. This model will appear in a forthcoming publication.

The results of these tests suggest that discrete solutions of copper and nickel are similarly acting at lethal levels to the zebrafish. Furthermore, these toxicants evoked supra-additive effects both in lethal and sublethal mixture levels. The extent of this supra-additive effect in hypothetically sublethal mixtures was, in some cases, death.

The authors propose that the apparent enhancement of potency in mixtures may be attributable to alterations in the kinetics of toxicant absorption, distribution, deposition, degradation, or excretion (see Fig. 1). This hypothesis is supported by the observations of Stokes (1975) who discovered that uptake rates of copper and nickel were mutually increased when the alga *Scenedesmus* was exposed to mixtures of these heavy metals.

ACKNOWLEDGEMENTS

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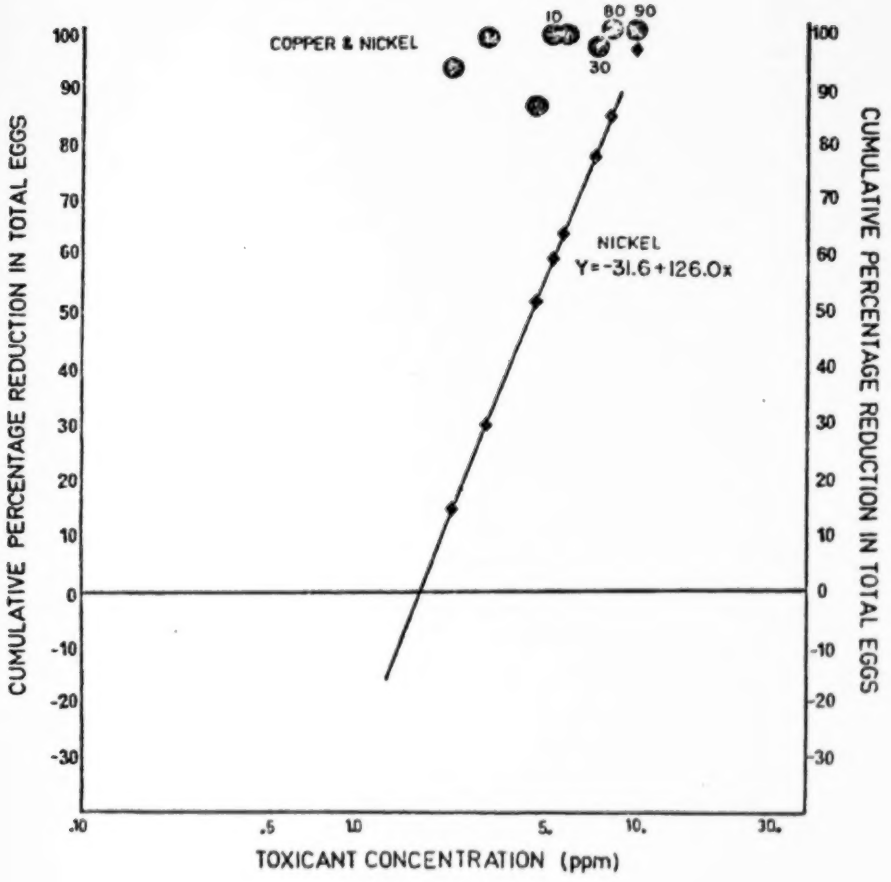

Fig. 6. 10-day sublethal responses of *B. rerio* exposed in a continuous flow-through system to mixtures of copper and nickel. Observed responses (in terms of percentage reduction in total numbers of eggs) to mixtures are depicted by dots and are plotted against C_A, the additive concentration computed by Equation (1). Numbers adjacent to dots represent observations of mortality ranging from 10% to 90% within the first 96 hours. Predicted responses, derived in accordance with the hypothesis of strict addition, are designated by diamonds on the nickel response curve.

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PHARMACOKINETICS IN RELATION TO TOXICITY ASSESSMENT

by

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ABSTRACT

Spear, P.A. and P.D. Anderson. 1978. Pharmacokinetics in relation to toxicity assessment. Proc. Fourth Annual Aquatic Toxicity Workshop. Vancouver, B.C., Nov. 8-10, 1977. Fish. Mar. Serv. Tech. Rep. 818.

Pumpkinseed sunfish, Lepomis gibbosus, were exposed to ambient solutions of copper sulfate in order to ascertain lethal toxicity and the associated pharmacokinetics of copper. The rate of copper accumulation in gills and the 96-hour LC50 approximated an inverse relationship as the magnitudes of the two variables changed with sunfish body weight. For rainbow trout, Salmo gairdneri, both variables were found to be independent of body weight. The results are discussed in relation to metabolic rate and the prediction of the tolerance of fish exposed to metal toxicants. Inter- and intraspecific examples are cited from the literature demonstrating the applicability of pharmacokinetics to the assessment of toxicity to fish, invertebrates, and algae.

Des crapets soleil, Lepomis gibbosus, furent exposés à des solutions ambiantes de sulfate de cuivre afin d'apprécier la toxicité léthale ainsi que la pharmaco-cinétique du cuivre. Le taux d'accumulation du cuivre sur les branchies et la CL50 après 96 heures se rapprochaient d'une relation inverse comme la magnitude des deux variables changeait avec le poids du corps. Dans le cas de la truite arc-en-ciel, Salmo gairdneri, les deux variables étaient indépendants du poids du corps. La discussion porte sur la relation des résultats avec le taux métabolique et la prédiction de la résistance des poissons exposés aux métaux toxiques. Des exemples inter- et intraspécifiques sont tirés de la littérature et cités en témoignage de l'applicabilité pharmaco-cinétique à l'évaluation de la toxicité aux poissons, invertébrés et algues.

INTRODUCTION

A first step towards determining the actual dosage received by an organism from the abiotic environment or via the food chain is to assay toxicant levels in the organism. Thus, Benoit (1975) demonstrated that the lowest ambient level of copper which resulted in copper accumulation into gills and liver was also the threshold for reproductive impairment in bluegills, Lepomis macrochirus. However, many authors have reported that internal concentrations of metal toxicants are not positively correlated with response magnitude (Bryan and Hummerstone, 1971; Brungs et al., 1973; Hodson, 1975; Jones et al., 1976, Solbé and Cooper, 1976). A more relevant assessment may be obtained from investigations of toxicant pharmacokinetics (i.e. the time course of toxicant uptake, accumulation, and elimination from specific organs or compartments).

Lloyd (1965) suggested that gills may be the critical organ for teleosts exposed to solutions of metal salts, and that factors which change the ventilatory volume passing over the gills would be expected to alter the rate of metal accumulation thereby influencing the lethal tolerance. Gill physiology, as indicated by the rate of oxygen consumption, and lamellar surface area may vary with the body weight of a fish (Winberg, 1961; Hughes, 1970). Also, many investigators have demonstrated that lethal tolerance may change with fish size (Carpenter, 1927; Goodman, 1951; Skidmore, 1967; Mount and Stephan, 1969; McKim and Benoit, 1971 and 1974; Benoit, 1975; Harrison, 1975; Spear and Anderson, 1975; Anderson and Weber, 1975; Howarth, 1976). The working hypothesis of this study was that the accumulation rate of copper by gills governs the weight-dependency of the 96-hour LC50.

METHODS

Representatives of the warm water species, pumpkinseed sunfish (Lepomis gibbosus), and the cold water species, rainbow trout (Salmo gairdneri), served as experimental organisms. Juvenile and adult fish were sorted into size classes by wet-weighing. The 96-hour LC50 was determined for each weight class. The lethal response studies were conducted according to standard procedures (Doudoroff, 1952; Sprague, 1969) using a serial dilution apparatus in a flow-through system. One year later, lethal response studies were repeated using a different sample of the sunfish population inhabiting the same pristine lake. To determine the accumulation kinetics of copper in fish gills, individuals representing certain weight classes of both trout and sunfish were exposed to solutions of copper sulfate. For each study, the concentration of the copper solution approximated respectively the mean LC50 for each species. The duration of exposure simulated the latent period prior to mor-

tality as determined previously. Groups of five fish were transferred to copper-free water at regular logarithmic intervals throughout the exposure period. Following a five-minute period, which allowed the rinsing of non-absorbed copper, individuals were immediately pithed and the total gill structure excised. Gills were prepared for analysis by acid digestion according to a procedure modified from Leonard (1971). Copper concentrations in water and tissue samples were determined by flame absorption spectrophotometry. The laboratory water was analysed regularly for factors which may modify copper's toxicity (Table 1).

Table 1. Analysis of laboratory water used in experiments.

		L. gibbosus	S. gairdneri
dissolved oxygen	% Sat	85 ±5	85 ±10
temperature °C	°C	21 ±1.0	10 ± 1.0
pH		7.2±0.2	7.8± 0.1
alkalinity	mg/l $CaCO_3$	85 ±2	85 ± 2
hardness	mg/l $CaCO_3$	125 ±5	125 ± 5

Both copper toxicity and accumulation rate data were expressed as functions of fish size according to the formula of Huxley (1950):

$$Y = aX^b \qquad (1)$$

where Y is the dependent variable, X is the organism's size, and values of the parameters a and b are calculated from the data. In the present studies, X is measured as the dried body weight.

Equation (1) is a quantitative basis for allometry (i.e. the growth of parts of the body relative to that of the body as a whole) and for changes in the rates of physiological processes during growth (Thompson, 1917; Huxley 1950; Adolph, 1949; and Kleiber, 1947 and 1975). With b equal to unity, the formula states that Y is proportional to body weight. If the value of b lies between zero and unity, the change in Y is disproportionate and less than the change in X. With b greater than unity, the change in Y is disproportionate and greater than the change in X. There is no weight relationship when b equals zero. In equation (1), the dependent variable is expressed on a per-organism basis, e.g. for metabolism, mg O_2/hr; however, when the dependent variable is expressed on a per-unit-weight basis, e.g. mg O_2/g/hr,

the following formula applies:

$$Y/X = aX^{b-1} \qquad (2)$$

which is a weight-specific relationship. The terms X^b and X^{b-1} are referred to as a weight factor and a weight-specific factor, respectively.

RESULTS

Allometric equations for the LC50 and the accumulation rate of each species were calculated from the values contained in Table 2. The 96-hour LC50 for sunfish was found to vary with body weight according to the equation:

$$\underset{\text{96-hour}}{\text{LC50 (ppm Cu)}} = 1.66\ W_{(g)}^{0.27} \qquad (3)$$

This relationship is presented graphically in Figure 1. The slope, 0.27, of the linear regression obtained by using a log-log scale is numerically equivalent to the weight factor exponent in equation (3). Stated simply, the relationship means that the LC50 for 2.0 g sunfish is greater than the LC50 for 0.2 g sunfish, but is not ten times greater.

For one weight class of sunfish, the time course of copper accumulation by gills is illustrated (Figure 2). Similar curves were plotted for the other weight classes. Rates of accumulation by sunfish, calculated for the 4-hour to 32-hour period of exposure, were found to vary with body weight as described by the weight-specific equation:

$$A\ (\mu g\ Cu/g\ fish/hr) = 0.0077\ W_{(g)}^{-0.35} \qquad (4)$$

where A is the accumulation rate of copper into gills. Equation (4) is presented graphically in Figure (3) to show the disproportionate decrease in accumulation rate of copper with body weight. The weight factors $W^{0.27}$ and $W^{-0.35}$ are similar in absolute magnitude which means that the LC50 varied as the inverse of the accumulation rate. In other words, the larger sunfish were more tolerant of copper poisoning and they accumulated copper more slowly at the gills. The relationship between the LC50 and the inverse of accumulation rate is shown in Figure 4.

Table 2 : LC50 and accumulation rates for *L. gibbosus* and *S. gairdneri* of various weight classes.

mean dry weight (grams)	96-hour LC50 (ppm Cu)	(95% fiducial limits)
	L. gibbosus - studies conducted in 1974	
0.24	1.24	(0.38 - 4.07)
0.47	1.30	(0.64 - 2.64)
1.00	1.67	(1.54 - 1.81)
1.85	1.85	(1.74 - 2.15)
	L. gibbosus - studies conducted in 1975	
0.43	1.24	(1.18 - 1.31)
0.63	1.66	(1.42 - 1.94)
1.32	1.74	(1.67 - 1.81)
	S. gairdneri	
0.94	0.20	(0.07 - 0.62)
6.76	0.19	(0.12 - 0.31)
46.0	0.21	(0.19 - 0.22)

mean dry weight (grams)	accumulation rate by gills x 10^3 (mgCu/g fish/hr)
	L. gibbosus
0.77	8.2
1.04	8.0
1.16	7.2
1.46	6.6
	S. gairdneri
2.1	1.3
6.7	1.6
23.0	1.2

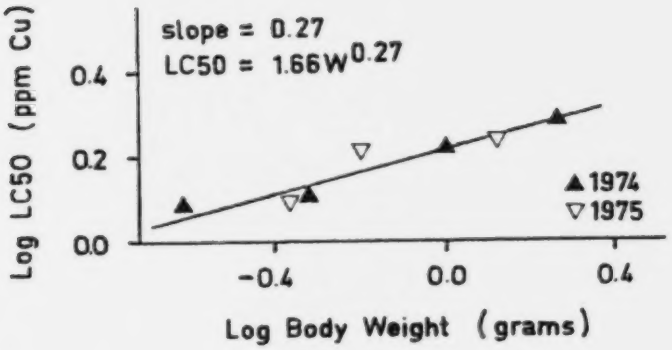

Fig. 1. The disproportionate change of 96-hour LC50 with mean, dried body weight of sunfish, showing that the slope of the regression line, 0.27, is the exponent of the weight factor $W^{0.27}$. Experiments were conducted in 1974 and were repeated in 1975.

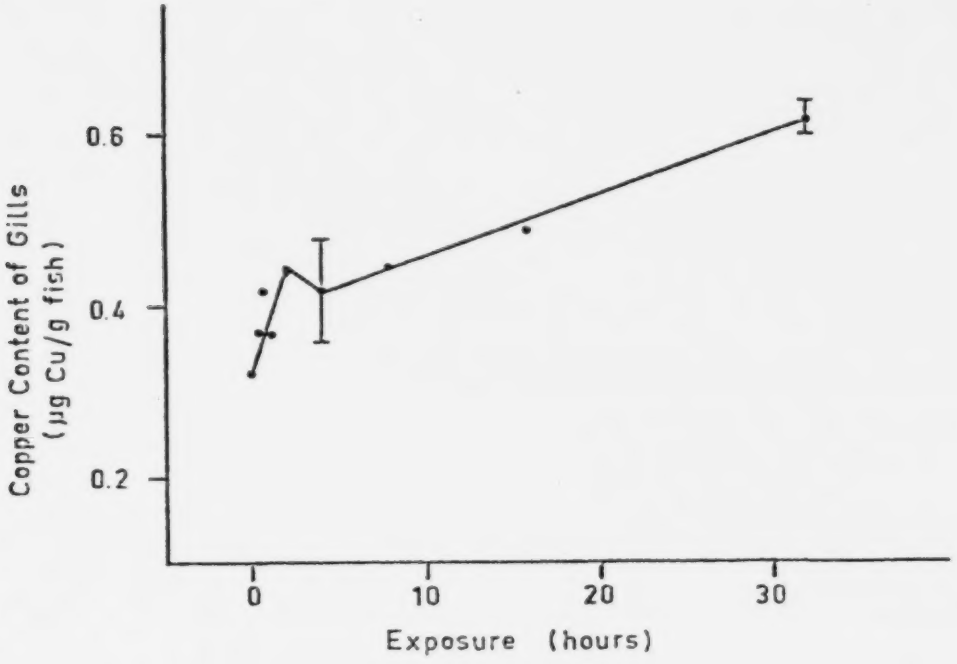

Fig. 2. The copper content of gill tissues for sunfish representing the 1.46 g weight class during exposure to copper sulfate. Standard deviations of copper content are shown for the beginning and end of the time period arbitrarily selected to monitor accumulation rate.

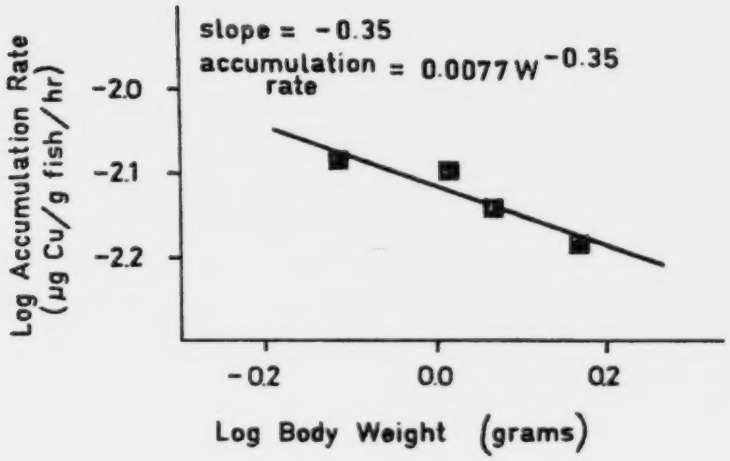

Fig. 3. The change of accumulation rate with mean dried body weight of sunfish showing that the slope of the regression line, -0.35, is the exponent of the weight factor, $W^{-0.35}$.

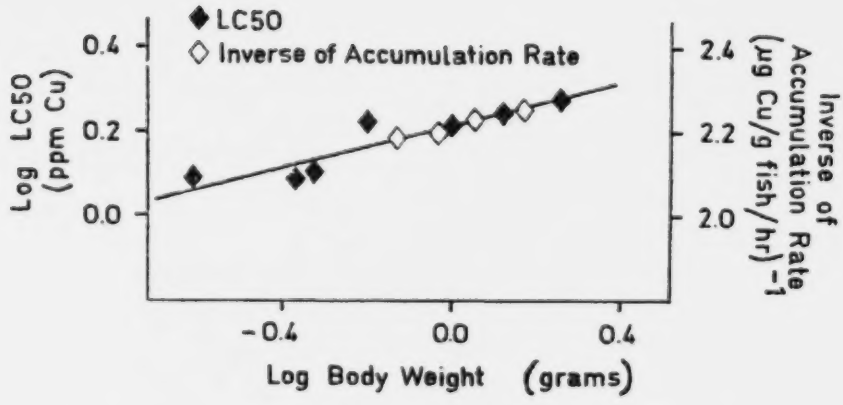

Fig. 4. The change of 96-hour LC50 compared to the change of the inverse of accumulation rate through a range in dry weight of sunfish.

In contrast to sunfish, the 96-hour LC50 for juvenile and adult trout was not found to vary with body weight, the weight factor being $W^{0.00}$. The accumulation of copper by gills is illustrated in Figure 5 for one weight class of trout. Curves were similarly plotted for the other weight classes. Rates of accumulation by trout, calculated for the 4-hour to 32-hour period varied slightly with body weight as described by the equation:

$$A\ (\mu g\ Cu/g\ fish/hr) = 0.0015\ W_{(g)}^{-0.05} \qquad (5)$$

The allometric relationships obtained for trout are essentially independent of body weight (Figure 6).

DISCUSSION

For both trout and sunfish, the weight-specific factor associated with accumulation rate was found to be the approximate inverse of the weight factor associated with lethal tolerance. The results were interpreted to mean that the accumulation rate of copper by fish gills governed the weight-dependency of the 96-hour LC50. The allometric equations describing accumulation rate are explained in part by the allometrics of metabolism for each species investigated.

O'Hara (1968) reported that the weight-dependency of metabolism for L. gibbosus was expressed by the weight factor $W^{0.71}$. The equivalent weight-specific factor, $W^{0.71-1.00}$ or $W^{-0.29}$, approximates the value $W^{-0.35}$ obtained in the present study for accumulation rate (equation 4). The similarity of these weight factors was interpreted to mean that accumulation rate varied as the metabolic rate for changes in the body weight of sunfish. Brett (1965) obtained the weight factor $W^{0.97}$ for active metabolism of the salmonid Oncorhynchus nerka. Job (1955) and Beamish (1964) found that the salmonid Salvelinus fontinalis has the weight factor $W^{1.00}$ for oxygen consumption. Rao (1968) demonstrated that weight factors for the metabolic rate of S. gairdneri may vary from $W^{0.78}$ to $W^{1.04}$ depending upon activity. Therefore, the metabolic rate of trout in the present study is assumed to have been proportional to body weight, with a weight factor of approximately $W^{1.00}$. The equivalent, weight-specific factor, $W^{0.00}$, concurs with that for accumulation rate by trout gills.

Oxygen diffusion across the gill surface is regulated, according to metabolic requirements, by changes in the ventilatory volume. The effective ventilatory volume determines the probability that a toxicant molecule will reach the gill surface (Lloyd,

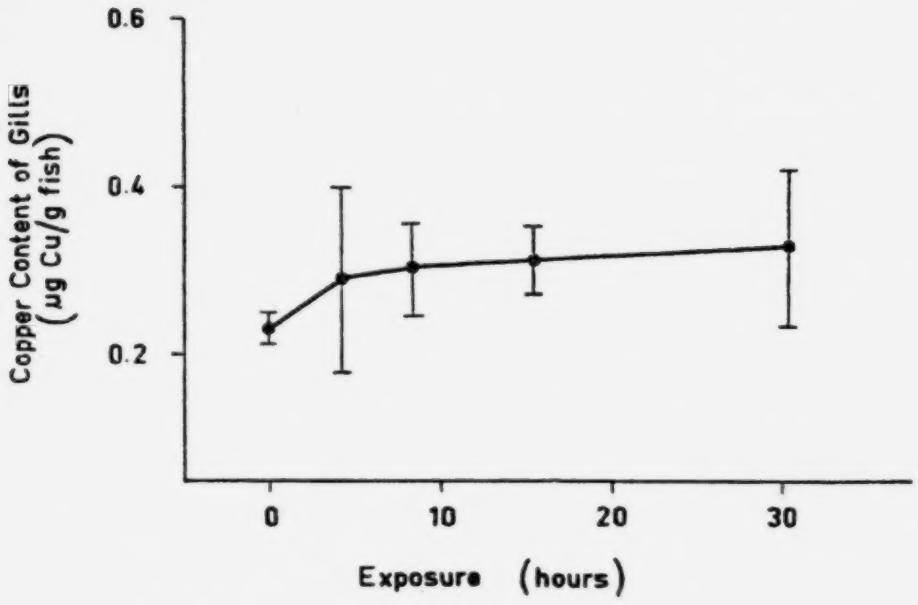

Fig. 5. The copper content of gill tissues for trout representing the 2.1 g weight class during exposure to copper sulfate. Standard deviations of copper content are shown.

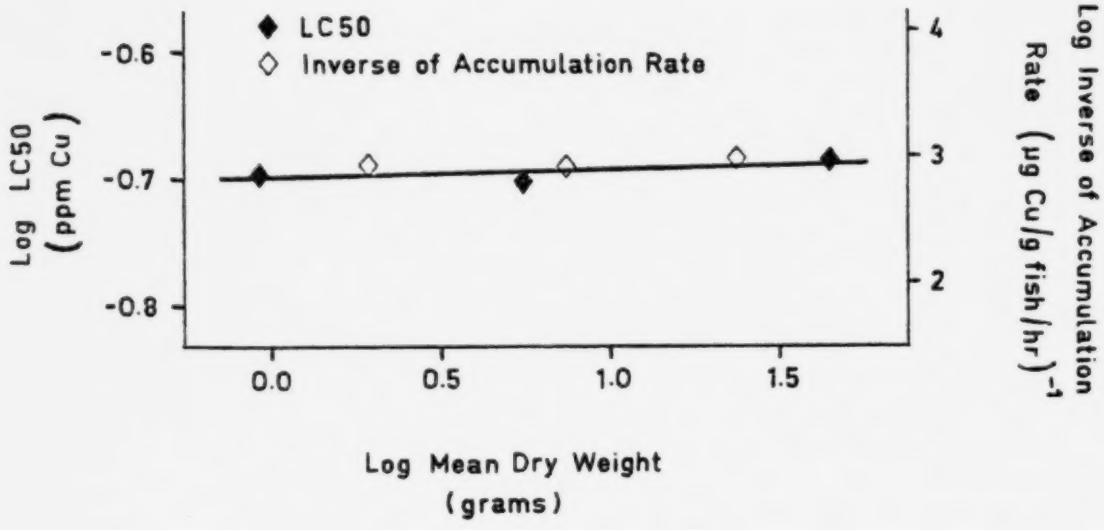

Fig. 6. The 96-hour LC50 compared to the inverse of accumulation rate for a range in dry weight of trout.

1965). Thus, metabolic rate may have governed the accumulation rate of copper in gill tissues. Accumulation rate may reflect the intrinsic activity of copper which was sufficient in certain individuals to cause a lethal response. Presumably, the larger sunfish were more tolerant of a given ambient concentration because the accumulation rate and associated intrinsic activity were less. The weight-independency of the tolerance of trout may have been governed by accumulation rate and the associated intrinsic activity of copper.

The results discussed above, in addition to previously reported relationships between fish metabolic rate and toxicity (Weiss and Botts, 1957; Lloyd, 1965; Herbert and Shurben, 1963; Skidmore, 1967), indicate that changes in lethal tolerance may be predicted on the basis of changes in metabolic rate.

Previous studies have shown that pharmacokinetics is a useful assessment of toxicity. A species of freshwater alga and a marine polychaete sampled from copper-contaminated areas were found to be more resistant to copper exposure than populations inhabiting pristine areas; and, resistance was related to lower rates of accumulation (Table 3). Several environmental factors have been shown to alter accumulation rate and an organism's tolerance to metal toxicants (Table 4). The chemical form of a metal toxicant is known to influence tolerance. Table 5 demonstrates that greater rates of accumulation of organic mercury and copper, and also a lower rate of accumulation of organically complexed copper, correspond with changes in tolerance.

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Note: The authors intend to publish a detailed report of this research at a later date.

Table 3 Biological factors which may influence accumulation rate and thereby change the response magnitude or LC50

author	experimental organism	metal toxicant	
Bryan & Hummerstone (1971)	polychaete *N. diversicolor*	copper	estimated rate of accumulation less in a resistant population.
Mierle & Stokes (1976)	algae *Scenedesmus* sp	copper	rate of accumulation less in a resistant population.
present study	sunfish *L. gibbosus*	copper	rate of accumulation greater and 96-hour LC50 less for smaller fish
present study	trout *S. gairdneri*	copper	no change in accumulation rate or 96-hour LC50 with fish size for juveniles and adults.

Table 4 Environmental factors known to influence accumulation rate and thereby change the response magnitude or LC50

author	experimental organism	metal toxicant	
MacLeod & Pessah (1973)	trout *S. gairdneri*	mercury	rate of accumulation greater and 96-hour LC50 less with increasing ambient temperature
Hodson & Sprague (1975) Hodson (1975)	Atlantic salmon *S. salar*	zinc	accumulation rate and mortality rate greater with increasing ambient temperature.
Mandelli (1969)	phytoplankton	copper	estimated accumulation rate and lethal response greater with increasing ambient temperature.
Mierle & Stokes (1976)	algae *Scenedesmus* sp	copper	accumulation rate and growth inhibition less in the presence of calcium.
Steeman-Nielsen *et. al.* (1969), Mierle & Stokes (1976)	algae	copper	rate of accumulation by *Scenedesmus* greater, and photosynthesis by *Chlorella* less, with increasing pH

Table 5 Different chemical forms of the toxicant which may influence accumulation rate and thereby change the response magnitude or LC50

author	experimental organism	metal toxicant	
MacLeod & Pessah (1973)	trout *S. gairdneri*	mercury (PMA, HgCl)	accumulation rate greater and 96-hour LC50 less with phenylmercuric acetate (PMA)
Stokes & Hutchison (1976)	algae *Scenedesmus* sp	copper	accumulation rate and inhibition of growth rate less with organically-complexed copper
Rathsack & Lohs (1969)	algae *Chlorella* sp	copper	accumulation rate and inhibition of growth rate greater with organically-complexed copper.

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APPENDIX I

DISCUSSION WORKSHOP

Explanation of the Procedure

In order to promote a free exchange of information, promote discussion and expose participants to varying points of view, we included three discussion sessions in the workshop program. The procedure was to hand out identical discussion topics to all participants and have them assemble in six small sub-groups to consider the topic and work out a response. Each sub-group elected a discussion leader and reporter. The discussion leader's job was to keep the conservation progressing and to draw the more reticent group members into the process. The reporter's responsibility was to record the findings of the sub-group and to present those findings when the entire meeting reassembled at the end of the session. A general discussion by the group as a whole followed.

Participants were randomly assigned to the sub-groups and sub-group content varied from session to session. This was done to expose participants to a variety of viewpoints and to break down cliques that might have formed had people from similar disciplines or organizations formed a sub-group together. In addition, this procedure allowed participants to get together in a more informal setting and promoted a free exchange of ideas as well as allowing participants to meet new acquaintances.

The discussion topics and related questions and instructions, as handed out to each participant follow, together with a sampling of each sub-group's reports and a general summary of the discussion. It should be noted that the findings of discussion groups and all positions given are not necessarily endorsed by Environment Canada.

Workshop Problem #1 - Wednesday, November 9

Guidelines - A workshop problem follows that is designed to stimulate discussion of sublethal and acute toxicity test data and its application to a receiving water situation. Proceed as follows:

- elect among your group a discussion leader whose job will be to keep the conversation going and involve all participants.
- elect a secretary who will record the major findings of the group and report the results following the discussion.
- proceed, using the flip chart to record findings in point form
- after 1 hour, reassemble in the Stanley Room where the secretary from each group will present results and a general discussion will follow.
- secretaries are asked to record findings in rough form on forms which will be handed out to each group. These findings will form the basis of published summaries of the workshops.

Problem

A variety of sublethal effect tests utilizing physiological, behavioral and histological methods with salmonids (mainly salmon and rainbow trout) exposed to kraft mill effluent were reported in the scientific literature. Many of these tests were conducted in soft, fresh water under standardized laboratory conditions. A few tests, conducted in saltwater with coho salmon, yielded similar results. Most of these results indicated that the sublethal incipient response threshold was between 0.1 to 0.05 of the 96 hr LC50. LC50's were determined using somewhat standardized procedures practiced by each laboratory and were generally of the static type with solution replacement every 24 hours with aeration, pH adjustment and effluent stored from 1 - 7 days.

A regulatory agency, in reviewing these results, recommended that an application factor of 0.05 of the 96 hr LC50 be applied as a suitable receiving water criterion for kraft mill effluent discharges. Consequently, regulations were prepared and promulgated to industry nationwide.

A large kraft pulp mill located on an estuary adjacent to a salmon river applied for a discharge permit. Their application stated that the average toxicity of effluent discharged had an LC50 equivalent to 50% full strength effluent when tested with rainbow trout underyearlings in freshwater. They proposed installation of a diffuser system that would achieve a dilution of at least 50:1 in the immediate vicinity of the outfall. The company's position was that fairly tight in-plant control and recovery, coupled with dilution at discharge would prevent damage to the estuary and its aquatic life. A moderate sized town (pop. 30,000) also discharges sewage to the area and there is a small contribution of agricultural waste chemicals (fertilizers, organic waste, some pesticides) from the river water shed.

Review the above information and discuss the following questions (do as many as you can).

1. Was the regulatory agency utilizing suitable logic in reviewing the literature and arriving at the criterion decision? What methods would have been better?

2. Were the bioassays and sublethal tests strictly comparable and could conclusions be reached from these data?

3. Was the regulatory agency justified in preparing a general criterion for application to both fresh and salt water nationwide?

4. Was the information on marine effects sufficient and, if not, what additional information was required?

5. Was the company's proposal sound and would you expect the treatment proposed to be adequate?

6. Neither the regulatory agency or the company proposed that any further assessment or monitoring was required. What related factors do you feel should have been considered and included in the regulatory agencies guidelines and the company's proposal?

7. What factors should be considered in designing and siting the outfall location?

8. Should discharge criteria be based on salmonid information or should some other information be included? What work must be done to define receiving water criteria? Is each discharge site and ecosystem a special case or are general criteria possible?

Response of Individual Discussion Groups to Workshop 1

Sublethal and Acute Toxicity Test Data and Their Application to Receiving Water Quality Considerations

Group 1 -

The group felt that insufficient data was present for the regulatory agency to reach a conclusion on a safe limit. There was a lack of information on estuarine organisms as most of the data was derived in freshwater utilizing salmonids under laboratory conditions. The group felt a variety of factors might affect the results of tests including variations in bioassay procedures, effluent handling and storage, loading density and interaction of salinity with toxic constituents. There was a feeling that a safe level of 0.05 96h LC50 was too high as it provided no safety factor. Further testing with resident species under natural conditions was recommended.

There was doubt about the conparability of sublethal test results which were utilized to derive the recommended criterion. It was felt that variations in procedure could produce misleading results if testing was not rigidly standardized.

Regarding the need for additional information, it was recommended that biological survey work in the area was desirable, including in situ sublethal testing with indiginous species at various salinities. Possible deleterious effects of sewage and other discharges would have to be considered in such a study along with chemical analysis of waste streams, consideration of water flushing and oceanographic features of the area, fish movement patterns and recreational usage.

Group 2 -

This group felt it was not scientifically justified to define receiving water standards using application factors derived from sublethal responses obtained in the laboratory under optimal conditions. Receiving water characteristics (temperature, salinity, D.O., circulation patterns, possible synergism with other toxicants, future waste loading) must be considered and incorporated in the criterion. Such a criterion does not provide sufficient safety margin for spills or effluent accumulation due to poor tidal flushing. Additive effects may become serious as various waste discharges from the mill, townsite and river increase with time. There was also a concern that toxicity may not be the major problem and the criteria were not directed at the other possible deleterious effects such as fish tainting and colour effects on phytoplankton productivity. Information on the chemical composition of the effluent was thought important in deciding on the nature of the hazard and whether additional treatment was required. It was recognized that simple dilution may not be sufficient if highly persistent and bioaccumulative materials were present in the receiving waters.

This group felt that careful consideration should be given to the biological resources frequenting the area. It would be important to decide what salmonid species utilized the estuary and how long they were resident there. Tests should be done with those species in salt water under natural conditions and major components of the salmon food chain should be included in the assessment. Damage to the estuary and its inhabitants should also be considered. Extrapolation of freshwater salmonid data to benthos, intertidal organisms and zooplankton are considered meaningless. An inventory of non-salmonid resources in the estuary and a well-designed monitoring program is recommended.

Group 3 -

Group 3 thought the regulatory agencies' approach to the problem was overly simplistic and did not include site-specific considerations. It was stressed that estuaries are considered extremely sensitive and important areas and siting of industries in such areas should be very carefully assessed if industrial usage of estuaries is tolerated. Criteria for estuarine areas should contain a good safety factor and consider food chain organisms as well as salmonids. Suitable protection should be present to prevent sublethal effects, tainting and coliform contamination. Investigative techniques used to derive criteria should involve the most sensitive sublethal test methods and critical life stages of test organisms.

It was felt that criteria based upon laboratory investigations were suspect and that such tests may not be strictly comparable. Even if the data quality and comparability were excellent, the group felt single species tests were inadequate. The use of toxicity standards and application factors may be necessary as a decision making tool but must be viewed as a minimum requirement which is certainly insufficient for estuaries.

Recommendations for increased information and an approach to the problem included a need to consider community structure and interactions, the influence of other comtaminants as well as mill effluent, possible in-plant treatment measures for mill and municipal waste, BOD loading and water circulation and quality considerations, hazard of chlorinated organics, chemical constituents of effluents, biological monitoring of the zone of influence, outfall siting, fish behavior and movement patterns—i.e.—a comprehensive site-specific study.

Group 4 -

This group felt the regulatory agency had utilized suitable logic in arriving at their criterion recommendation. They felt however, that literature reviews should be used with a lot of qualification and should include specific bioassays in kraft mill effluent in the laboratory, new bioassay data and considerations of specific effluent characteristics, water quality and local fauna and fisheries resources when applied to a given area. They felt that laboratory-based studies alone were insufficient and that firm conclusions for direct application to receiving waters were not valid unless they were varified by site-specific in situ testing. There was a feeling that the regulatory agency was justified in preparing a general national criterion, with the above qualifications about its

applicability, but there was concern about comparability of data on fresh and salt water organisms. The group felt the companies' position was sound based on their interpretation of the regulations, but that the regulatory agency should respond by providing more site-specific information.

Group 5 -

The group as a whole felt the regulatory agency was not justified in arriving at a criterion and that more testing should have been done prior to making a decision. It was recommended that sub-categorizing kraft mill effluents based on the industrial process used might be useful and that additional tests with lower trophic levels, especially marine species were required. Although the tests described were not considered strictly comparable, it was felt that conclusions could be reached but that these conclusions were not applicable to all industrial processes. It was agreed that the agency was not justified in setting national standards for fresh and salt water and that knowledge in relation to marine species was inadequate. Tests with various trophic levels were required and it was important to investigate the relationship between LC50's and sublethal effects as affected by water quality.

A majority felt the company's proposal was sound but several felt dilution was not proper treatment and that more emphasis should be placed on best available treatment technology. The use of freshwater as the dilution water and a freshwater organism for LC50 determination of an effluent discharging to an estuary was considered inappropriate. Recommendations on the need for site-specific information on the water quality, biota and trophic relationships were similar to other groups. It was recommended that a zone of passage for fish and protection of at least a portion of the estuary be considered.

Group 6 -

This group felt that national regulations should be developed in stages, ie

Stage 1 - effluent standards based on:
a) compliance schedules
b) best practical technology
c) volume and type of industrial production
d) effluent chemical characteristics
e) effluent monitoring programs

Stage 2 - Site-Specific considerations such as: sublethal effects - (variety of organisms required):
avoidance
tainting
colour
thermal effects
bioaccumulation
osmoregulation, etc.
synergistic effects of other discharges

This group felt that the estuary was a special situation and should be considered in a site-specific way. There was not enough basis in the regulatory agencies' approach to the problem and information of the "stage 2" type above was required. Question arose as to whether this could be practically achieved in each case and disagreement occurred on the nature of the site-specific study required. Permit requirements have to be defensible in a legalistic sense and supported by adequate data. It was recommended that a safety factor should be added to the 0.05 96hr LC50 criterion.

Overall Summary of Workshop 1

In observing the progress of the discussions and the points of view summarized above, some interesting generalizations can be made of the group's views on receiving water standards and utilization of laboratory data. These are:

- Scepticism exists over the overly simplistic approach to deriving general receiving water standards from laboratory information with specific organism types (i.e. - salmonids).
- Doubts are present as to the comparability of laboratory bioassay test results and sublethal testing techniques reported in the literature.
- Application of freshwater organism test data to the marine environment is a questionable practice and is not scientifically justified.
- There is a strong feeling that site-specific information is required on a broad basis which includes information on physical, chemical and biological processes as well as on important trophic relationships.
- The topic of synergistic effects and joint action must be addressed in receiving waters, particularly in instances where individual effluent streams tend to increase in volume flow with time.
- Dilution is generally not viewed as a solution and use of in-plant treatment as well as dilution is advocated.
- Criteria such as the 0.05 96h LC50 value for kraft mill effluent should have an additional safety factor built in to allow a margin of safety for spills, effects on sensitive organisms or life stages, etc.
- Criteria should be defensible in a legalistic sense and be backed with hard data using indiginous species tested under site-specific conditions.
- Effluent criteria may be considered a minimal requirement for promulgation on a national basis but the overall assessment of a discharge should not be based wholly on such criteria but also upon consideration of site-specific concerns.

The preceeding discussion is an interesting one as it centers upon a major national problem facing regulatory agencies. First, such agencies are charged with the responsibility for deriving criteria for use as effluent or receiving water standards, yet in many cases the data base is very poor. Frequently,

freshwater information is used to derive marine criteria which may be a very questionable practice. There is a very strong feeling among most participants that receiving water considerations can only be met with good, site-specific information. The immediate problem, of course, is that such assessment is often not practical in every case and may not be economically possible in many cases. This suggests that the onus is on us, as aquatic researchers, to develop sensitive and meaningful test protocols and practices that can be used in an expeditious way to at least make reasonable judgements as to the hazards of specific discharges. It is idealistic to expect that we shall obtain all the information necessary in every case but perhaps we can at least work towards improving our assessment procedures in the hope of making more realistic recommendations in specific instances.

Workshop #2 - 1000-1200 hours, Thursday, November 10, 1977

During operation of a copper mine a large underground water body was encountered which required round-the-clock pumping to keep the mine shaft from flooding. Approximately 30,000 gal/day is continuously discharged to a small, naturally formed depression in the land near the mine site. The mine water thence flows to a small stream about 0.5. mi. distant and, in the process, scours the sedge-like vegetation mat along its course. The stream averages about 50 feet in width and provides an excellent salmonid recreational fishery. Spawning beds are common throughout the length of the stream in the general vicinity of the mine site. Assorted assemblages of other fish species and invertebrates make up the stream community. The stream is ice-covered about 4½ months of the year.

The mine water is known to contain 4 - 15 ppm copper with undetermined quantities of Zn and Pb. The copper appears to be mainly in the form CuS and some Mo may also be present. In addition, the mine water is salty and has an apparent "salinity" of 1-2 percent.

On several occasions dead fish have been observed downstream from the mining area. In three months, the mining company faces court proceedings for contravening the Fisheries Act. If contracted to direct a team to assess the probable impact on the stream receiving waters for the purpose of presenting evidence in court, how would you proceed with the scientific investigation?

Response of Individual Discussion Groups to Workshop 2

Design Of A Field Program To Assess The Impact And Hazards Of Mine Wastes Discharged To A Stream Environment

In this case, not all reporters handed in the results of their discussions. Here is a synopsis of the findings of four discussion groups:

Group 1 -

This group made the assumption that they were an independent contractor working to provide evidence for consideration by the courts. They assembled a study team and included legal expertise to ensure that the approach and findings were defensible in the courtroom. The major evidence to be used in the case was considered to be the occurrence of dead fish which indicated a toxic condition and discharge of a "deleterious substance" in contravention of the Fisheries Act. A two month, mid-summer investigation period was selected with the remaining time to be devoted to data processing and reporting.

The proposed research format was:

1) To determine the physical and chemical propertics of the effluent discharged, and of water in the depression and at its confluence with the stream at 4 sampling periods(including metal content, hardness, pH, temperature and presence of other possible contaminants).
2) The zone of influence of effluent on the stream would be mapped utilizing bacteriological methods (standard plate counts) and dye dilution studies.
3) The chemical, physical and biological properties of the stream would be characterized with respect to water quality, fish habitat utilization and seasonal effects of interfering substances - ie. - sedimentation. Some local environmental information and archival records on the stream were presumed available for consideration. Studies were to take place upstream from the mine effluent discharge, at the discharge site and downstream from the discharge every two weeks during the experimental period. Biological survey techniques included:
   - analysis of tissues of resident species for copper content
   - species inventories and community structure
   - "routine" assay techiques:
     - in situ bioassay cage studies
     - Daphnia bioassays
     - algal growth rate studies
     - histological analysis of fish gills
     - avoidance/preference studies in salmonids
     - study of effects of metals on smoltification in young salmon

(The group felt that a clearer definition of the above "routine tests" was required - some may not be legally defensible because of conflicting results or the possibility of challenge by expert witnesses).

It was envisioned that the above approach would provide good evidence on the nature of the toxic hazard and impact of a possible discharge of a deleterious substance, especially if combined with historical data on the stream prior to pollutant discharge. If possible, recommendations on alternate effluent discharge and treatment possibilities would be made in the report.

Group 2 -

This group made the realistic assumption that manpower shortages and funds would limit the investigation team to two individuals over the 3 month period. They felt the following investigative regime could be followed:

- collection of mine water and receiving water samples for thorough chemical analyses
- determine physical parameters at point of discharge and in stream receiving mine wastes
- conduct laboratory 96h LC50 tests on effluent with salmonids and consult the literature
- conduct in situ bioassay toxicity studies with salmonids at 6 - 7 locations in the stream with an exposure duration of at least 1 week
- as effluent may prove non-toxic, it was considered useful to look at any mine monitoring data available and consider natural conditions such as variations in stream flow, effluent dilution, etc.
- chronicle efforts by taking lots of photographs and maintain continuity of evidence
- attempt to have remedial action taken by industry to install suitable treatment facilities following the case.

Group 3 -

Group 3 assumed that a charge was laid against the company under Section 33(2) of the Fisheries Act. Their goal was viewed as supporting the charge by demonstrating the deposit of a deleterious substance into water frequented by fish. The discussion took the position that the entire study was aimed at supporting the prosecution of the case by:

- documenting the presence of fish in the area and demonstrating that fish utilize the area for feeding, spawning, etc.
- documenting of evidence on fish kills in the system.
- involving expert witnesses to provide testimony on the impact of the discharge on the stream ecology
- involving experts in performing acute LC50 tests and in situ bioassays to provide toxicological evidence on the hazardous nature of the discharge
- critical factors were defined as continuity of samples and evidence; the necessity to link the mine to the discharge (chemical and engineering evidence); quality of expert witnesses, establishing the toxicity of the discharge

Group 6 -

A competent field survey was envisioned to determine the nature, source and extent of damage to the stream system which involved:

- a survey of benthos in one characteristic habitat type, noting species make-up in relation to effluent sensitivity. Community changes in relation to the source of stress would be documented.
- a chemcial survey would be conducted to examine and trace known contaminants in water and accumulative metal build-up in tissues of stream-dwelling organisms (relate to permissible contamination limits for fish tissues).
- caged fish tests in the depression receiving the mine effluent together with such tests in the stream would establish harmful effects of the effluent on salmonids.
- utilize the appropriate wisdom, literature and common sense in designing and interpreting the results of the study.

Overall Summary of Workshop 2

This discussion session was rather fascinating in that two general trends emerged in the way that participants responded to the question. One trend was to interpret the situation and the response required in a legalistic way - ie - what evidence was required and what must be done to achieve a prosecution? Persons choosing that rationale tended to limit their study recommendations to items critical to establishing a deleterious condition for fish and the necessary steps to support such a contention in the courts. The other view supported very detailed and complex field studies, often utilizing a variety of methods obviously labour-intensive and expensive to carry out. Frequently, there was heated dissention among participants as to what should be done. It would appear that some people would like to measure everything while others chose to emphasize certain types of studies, possibly related to their own professional expertise and experience.

There is probably no single "right way" to approach a problem such as the one described and alternate methods of investigation are open to the researcher, a number of which might yield useful results. Often the big limitation in such instances is available expertise, money, manpower and sometimes, time. It is evident that we do need a variety of well established test regimes and methods for site-specific useage. The dissention among advocates of the various techniques shows that we are far from achieving standardization or agreement among investigators as to methods of choice. It would appear that use of a good deal of wisdom and common sense, coupled with experience as to what type of information is legally defensible, is the best approach to complex environmental assessments.

WORKSHOP #3 - THE FUTURE - Goals and Shortcomings PM - Thursday, November 10

This workshop is intended to explore the future in an attempt to ascertain whether we are following the right pathway in aquatic toxicology studies and practices. A number of questions are posed - do not feel you have to address all of them - choose some which appeal to your group. Try to spend a few minutes on Part B. If you wish to discuss any other issue go ahead.

Part A - Future Issues

1. In reviewing "Water Quality Criteria, 1972", the U.S. EPA bluebook, John Sprague pointed out that many receiving water criteria proposed were somewhat arbitrary and based on weak data. Only a few types of tests (reproductive tests, tests with animals with short life cycles, *Daphnia* and algal tests) appeared productive. Is much of our work of little value and are we wasting effort and money on certain types of work which may not prove particularly productive?

2. In Canada and the U.S. we base many of our decisions regarding the hazards of toxic substances on somewhat limited information, often pertaining to commercially important species. Frequently freshwater findings are extrapolated to marine situations. Is this a realistic approach and what better strategies are required?

3. Due to the large volume of effluent required and the intricacies of technique, it is proving very difficult for many laboratories to utilize continuous flow bioassays for routine toxicology work - especially with industrial effluents. Thus there is a move towards simplifying procedures and utilizing simple static-type tests. What are the advantages and pitfalls of this philosophy?

4. What progress is being made in deriving useful marine bioassay tests and test organisms for routine toxicological or impact assessment techniques? Are marine larvae (fish or invertebrates) really useful and practical and what areas of research must be emphasized?

5. Is a national bioassay procedure with a standard test organism (rainbow trout) and prescribed limits for test variables such as temperature, pH, fish size etc. really practical and justifiable for use across the country without regional modification? How well does such a test reflect the sensitivity of aquatic organisms in a site-specific sense?

6. Are the results of toxicity tests (acute and chronic) found in the literature comparable in any real sense and are adequate physical and chemical measurements being made to complement such results? Is our knowledge of water chemistry sufficient to deal with difficult situations such as joint toxicity, complexation, salt water interactions etc?

7. What do you feel are the major challenges facing aquatic toxicologists in the years to come? What pollutants and questions are of the highest priority?

8. What is the value of in situ testing and how should this type of test be expanded or refined?

Part B - Future Toxicity Workshops

1. Should the Toxicity Workshop continue to be an annual event and is it a useful meetin
2. How would you suggest we improve our meetings and what format would you like to see?
3. What topics might be tackled next time?
4. What shortcomings have the present and past meetings experienced?
5. where should we hold the next meeting?
6. Do you favour changing the meeting to the springtime to avoid conflicts in the fall with other toxicity meetings?
7. What do you think of incorporating our group into an official society with newsletter, etc?

Workshop 3

A) The Future - Challenges to Aquatic Toxicology
B) Needs For Forthcoming Aquatic Toxicity Workshops

Most of the groups responded to Part B of this workshop only and did not have time to consider the questions in Part A. Two groups did address some of the issues in Part A and a summary of their findings follows.

Question 5 -

A white- rat-type reference technique is valuable provided that conditions can be suitably standardized. It is important to have a legally defensible technique that is recognized by the courts through repeated usage.

Question 6 -

Results of acute and chronic toxicity tests found in the literature may not be strictly comparable due to differences in technique, test organisms, test water etc. The necessary knowledge of water chemistry is frequently not available or properly investigated and causes of variation are often not recognized.

Question 7 -

Major challenges facing aquatic toxicologists and priority pollution concerns include:

- the need to take laboratory information to the field and establish viable field techniques
- understanding the various mechanisms of toxic action
- more emphasis should be placed on industrial effluent work rather than on work with pure solutions under laboratory conditions
- pulp and paper studies should include an evaluation of stimulative effects on ecosystems as well as negative effects
- specific effluents require characterization (ie - drilling muds). Petroleum hydrocarbons, fluoridation and halogenated organics require investigation.
- emphasis must be placed on technology development in laboratory and field
- results have to be extrapolated to the real world-discrete impacts such as sublethal effects must be understood. What do they mean? What are the population survival implications?
- organic analysis must be emphasized in water pollution studies and sophisticated techniques utilized to understand the nature and hazards of complex organic effluents

Question 8 -

The difinition of in situ testing requires clarification. It could relate to benthic surveys, cage studies of toxic effects (acute or sublethal) or the use of a laboratory on site. Such tests can be used to achieve specific objectives which could not be studied or conditions properly simulated in the laboratory otherwise.

Part B - Structure of Future Workshops

Group 3 -

The toxicity workshop is considered a very worthwhile effort which provides for an informal contact for interchange of information, both current and for the future. Formality should be avoided and a society concept is not endorsed. The open forum with open attendance is highly desirable and the current size of the meeting should be retained, if possible. It is desirable to hold the meetings in different parts of Canada annually, preferably in the fall, and such a meeting might alternate between 6 established locations in the country. The theme of the meeting should be broad to incorporate varied interests but workshop sessions should have narrow, easily-discussed topic areas (eg. - loading densities and how they affect toxicity tests or selection of a marine invertebrate suitable for toxicity testing). Suggestions for future sessions:

a) workshops with free discussions of selected topics (possibly preceeded by invited speaker or paper)
b) panels - with outside expertise to stimulate free discussion. Group discussion followed by production of "a state of the art" summary.
c) large topics for meetings might include:
   1) Attitudes of industry and regulatory agencies toward toxicity methodology (include the Canadian forest industry, economics, legal considerations).
   2) Assessment of current methodology and needs for better methodology. What tests are good that are available and where are the weaknesses?
   3) Design of field studies. What do we do following an oil spill?

Group 4 -

The Aquatic toxicity workshop is desirable and should be repeated. There should be three days of meetings with less emphasis on tours. It is desirable to confine the proceedings to one or two selected topics. A workshop could be utilized at luncheons. Newsletters, formality, formation of a society and anything which destroys the informality of the meeting is discouraged. The fall timing of the meeting is desirable. Possible topics for discussion:

a) Mock trials - two viewpoints possible:
   - Industrial view that we should be working together to improve the environment.
   - Government view that it is important that the court process be understood by all.

b) Emphasis on the strategies and usefulness of utilizing aquatic toxicology for monitoring.
c) Consideration of organic contaminants problems.
d) Implications of fish contamination as a public health hazard

Group 5 -

This group felt that the meeting was worthwhile. Two day meetings were considered optimal. They felt there was a need to draw on a wider community,

particularly industry,and develop an international framework for exchange of information. More ecological expertise and animal behavior specialists should be involved and increased emphasis should be placed on ecosystem assessment. Engineers should also be involved to develop a "team" approach to problems. Panel discussions should be avoided and there should be increased emphasis on workshop-type discussion groups. A list of participants and affiliation list should be distributed at the start of proceedings to facilitate introductions and informality. Tours should include visits to industrial pollution sites.

Regarding program content, there should be more emphasis placed on implications than on methodology. Involvement of ecologists would enhance an understanding of implications. There should be an attempt to identify areas of interest among the participants to be used in the selection of workshop topics. Possible topics might include:

a) drilling muds and their components
b) prosecution procedures - legal constraints
- adversary relationships
c) refinement of testing methods and toxicological assessments
d) methodologies

Group 6 -

The Aquatic Toxicity Workshop is worthwhile and should continue. It is desirable to keep the current discussion workshops and make the proceedings less "conferency". At least one good review paper should be given annually and inclusion of marine material is highly desirable. Two days are suitable for meetings and the Wednesday/Thursday format is good as Tuesday and Friday are useful for tours, visiting labs, travel etc. Papers may tend to be too formal at the meetings with detailed descriptions of methods instead of emphasis on "news". Workshop discussion groups are desirable but choice of topic is critical. The group disliked the idea of a society and wished to encourage informality. There was considerable discussion of the need for an aquatic toxicology journal and some displeasure was expressed over the Journal of the Fisheries Research Board's editorial policies regarding publication of environmental papers.

Desirable topics for discussion at future meetings could be:

a) environmental law, mock-legal proceedings, involvement of lawyers
b) maintain emphasis on new techniques
c) prevention - methods of removal of contaminants before they enter the water.

Overall Summary-Workshop 3

It would appear that a majority of participants are convinced the Aquatic Toxicity Workshop is a worthwhile Canadian forum and that it should continue on an annual basis with a fall meeting of at least 2 days. It is obvious that informality of discussion and format is the preferred structure and participants are unanimously opposed to a more formalized society concept or growth of the

meeting in size. People favour holding the meeting in various parts of the country and alternating locations to facilitate attendence and allow for visits to other laboratories and work environments. Small discussion groups tackling discrete problems are encouraged as they promote interchange of ideas, add informality and allow new friendships to be formed. Interest in various topic areas seems to be as diverse as the participants' work locations, expertise and emphasis, hence it will probably always be difficult to satisfy everyone as to program content.

Some thoughts on our Committee's approach to organizing the meeting might be useful for future program organizers:

1) We envisaged several discreet themes for the meeting -ie- acute and sublethal test methodology and applying results to the environment. The problem however, is fitting the submitted papers to such a theme as people tend to work on discrete, often method-oriented topics, which may be hard to incorporate in a given theme. Possibly, a theme can be better developed by the use of workshops or invited papers, with a general contributed papers section to cover other topics.

2) There was no problem soliciting sufficient papers - we received over 30 abstracts and selected approximately half of them for presentation at the meeting. The big problem comes in obtaining and referreeing papers for publication following the meeting. Delay is inevitable and proceedings are held up.

3) We chose a location for the meeting which was central to the city and various laboratory sites to facilitate tours and sightseeing. Luncheons and banquets were included to prevent the group from breaking up and dispersing and in order to promote informality.

4) Workshop topics were chosen to appeal to diverse interests and talents and were purposely not pre-circulated to the participants. We felt the discussions should be spontaneous and "off the cuff" and that concepts and approaches were more important than the detail which would have resulted had people researched the topics beforehand.

5) All costs of luncheons, room rentals, transportation, coffee etc. were included in the registration fee. We broke even on the costs of the meeting, excluding publication and mailing costs, which were shared among sponsoring groups. It is recognized that registration fees are a problem for some participants and future meetings should explore ways of covering costs. A problem that immediately developed was one of bank accounts and money handling. As the group is not incorporated as a society, money had to be handled on a personal account basis with resultant risk to the treasurer re N.S.F. cheques, cost over-runs on meals etc.

6) Pre-registration was a partial success. We had a total of 123 registered participants, 104 of which pre-registered and 9 of which both pre-registered and prepaid. A total of 63 hotel rooms were occupied, many on a shared basis. Some additional non-registered persons sat in on various sessions and it was estimated that approximately 160 persons attended some of the general sessions.

7) A poster session where participants submitted graphics and visually-attractive displays was included although response was disappointing. This was an attempt to allow people not presenting papers to display material which would encourage feedback and discussion with meeting participants. Such displays can be very useful but require a lot of work on the part of the person presenting the display. Posters were presented by:

Norman Bermingham - E.P.S., Longuenil, Quebec
Evaluation of the relative toxicity of water and wastewater using an algae, *Selenastrum capricornutum*.

Joseph Heald - Case Existological Laboratories Ltd., Victoria, British Columbia
Engineering support for the CEPEX study.

J.W. Kiceniuk - Fisheries and Marine Service, St. John's, Newfoundland.
Site and mode of action of detergents on fish.

J.C. Davis
G.L. Greer
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